

**The importance of jackals and domestic dogs for the  
transmission of generalist canid pathogens to  
sympatric carnivores in Namibia**

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## **Declaration**

**I declare that the research described within this thesis is my own work and that the thesis is my own composition.**

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Edinburgh, 2004***





**A juvenile black-backed jackal (*Canis mesomelas*),  
Cape Cross Seal Reserve, Namibia. S. Gowtage.**



Black-backed jackals fighting at the carcass of a Cape fur seal (*Arctocephalus pusillus*), Cape Cross Seal Reserve, Namibia. Courtesy of G. Vila-Garcia.

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## Abstract

Generalist canid pathogens are of increasing concern to carnivore conservation. The continued encroachment of rapidly growing domestic dog (*Canis familiaris*) populations into wildlife areas provides increased opportunities for disease transmission between dogs and both terrestrial and aquatic wildlife species. The jackal species, widespread throughout sub-Saharan Africa and adaptable to both human-disturbed and protected landscapes, are thought to play a central role in the maintenance and transmission of generalist canid pathogens and to act as a source of infection for both wild and domestic species. This thesis investigates the role played by the black-backed jackal (*Canis mesomelas*) in the transmission and maintenance of canine distemper and other canid pathogens within a Namibian coastal carnivore guild, comprising black-backed jackals, brown hyenas (*Hyaena brunnea*), Cape fur seals (*Arctocephalus pusillus pusillus*), and domestic dogs from urban settlements.

The first of the chapters containing original data, Chapter 3, describes a canine distemper outbreak in the jackals and dogs of the guild, providing the first evidence for, and a description of, natural canine distemper infection in jackals and demonstrates that this species was responsible for the rapid spread of the epidemic along the Namibian coast and for the spill-back of the virus into the dog population. Chapter 4 investigates the exposure of the sympatric Cape fur seal population to morbilliviruses using virus neutralisation tests for canine distemper, phocine distemper and dolphin morbillivirus and demonstrates that it is unlikely that a morbillivirus is endemic in this population and that the seals did not suffer a large increase in seroprevalence or mortality as a result of the canine distemper outbreak in the jackals and dogs. In Chapter 5, serological data indicates that jackals and dogs both have high levels of exposure to canine adenovirus, canine herpesvirus and sarcoptic mange but that exposure to canine parvovirus, although high in the dogs is very low in the jackal population. Canine adenovirus and sarcoptic mange are likely to be endemic in the jackal and dog populations, hence jackals may act as a source of infection to sympatric wildlife and as a source of re-infection for dogs. In Chapter 6, behavioural observations of jackal-jackal and jackal-seal interactions are used to determine contact rates for the transmission of canine distemper virus. Contact rates of different subsets of the jackal population are compared to determine if there is any heterogeneity which would support the existence of a core group of individuals primarily responsible for the spread of canine distemper virus within the jackal population. Contact rates and overlap of jackal home ranges at the colony, as determined from radio-telemetry data are used to help understand the observed prevalence of exposure to canine distemper virus in the jackal and seal populations.

The results of this study are discussed in relation to the control of distemper infection in wild and domestic canids, in Namibia and elsewhere in sub-Saharan Africa, and the importance of disease surveillance in jackal populations is stressed as disease management programs aimed at controlling canine distemper and other common viral infections in domestic dogs must assess the risk of re-infection from jackals or other sympatric wildlife species.

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## Abbreviations used

CAV-1	Canine adenovirus type 1
CCS	Critical community size
CCSR	Cape Cross seal reserve
CD	Canine distemper
CDV	Canine distemper virus
CHV	Canine herpesvirus
CNS	Central nervous system
CPV	Canine parvovirus
CPV-2	Canine parvovirus type 2
CVL	Central veterinary laboratory, Windhoek
DMV	Dolphin morbillivirus
DRC	Democratic resettlement community
ELISA	Enzyme-linked immunosorbent assay
HAI	Haemagglutination inhibition test
IUCN	The world conservation union
JHE	Joint hypergeometric maximum likelihood estimator
MV	Morbillivirus
NCD	North central division
PDV	Phocine distemper virus
PMV	Porpoise morbillivirus
RT-PCR	Reverse transcription PCR
VN	Virus neutralising
VNT(s)	Virus neutralisation test(s)
SPCA	Society for the prevention of cruelty to animals

## **Chapter 1: General Introduction**



The assessment and control of disease risk to wildlife is one of the top priorities in conservation research. Most of the pathogens which cause human and animal diseases are multi-host, microparasite pathogens with a broad host spectrum which may be maintained by different domestic or wildlife hosts under different epidemiological circumstances (Murray *et al.*, 1999, Daszak *et al.*, 2000, Cleaveland *et al.*, 2001, Taylor *et al.*, 2001). The design of effective control measures for multi-host pathogens is dependent upon the identification of the reservoir hosts and the mechanisms of persistence in the reservoir populations (Haydon *et al.*, 2002). But the dynamics of multi-host pathogens in wild animal populations are complex and the reservoirs are rarely identified (Haydon *et al.*, 2002). In many cases it is not known if a pathogen disappears from the host population between epidemics or whether it persists at very low levels (Dye *et al.*, 1995). Whether and how multi-host pathogens persist, and if so, which hosts are acting as reservoirs, are some of the pivotal questions in infectious disease epidemiology today (Cleaveland & Dye 1995, Dye *et al.*, 1995).

### **1.1 Pathogen persistence and the critical community size**

A pathogen may only persist in a host population if two general conditions are met. Firstly, an infective individual must transmit the pathogen to, on average, one or more susceptible hosts. In epidemiological terms, the basic reproductive rate ( $R_0$ ) of a pathogen in a population must be greater than or equal to 1; if the number of new cases per infective individual drops to below 1 then the pathogen will die out (Anderson & May 1991). Thus there is a threshold population density,  $N_T$ , below which pathogens will not persist (Anderson & May 1991).

Secondly, the pathogen may also die out if the rate of spread through the host population is too great: it will run out of susceptible hosts to infect, when all those that can be infected have either died or become immune. This means that a pathogen will require an influx of new susceptible individuals (by birth,

immigration or loss of immunity) at a rate sufficiently high to balance the removal of susceptibles as the pathogen spreads through the population (Dye *et al.*, 1995). This requirement can be translated into a 'critical community size' (CCS) i.e. the minimum host population size (and density) required to allow the pathogen to persist at the population level (Bartlett 1960, Tompkins *et al.*, 2002). Many microparasitic (viral) infections such as measles cannot persist in the environment and are highly contagious. This results in a large CCS for persistence so that the hosts may give birth to new susceptibles at rate that keeps pace with their loss by infection (Anderson & May 1991); the CCS of measles has been estimated at between 250,000 and 300,000 people (Black 1966).

If this threshold CCS size is not met then the pathogen may fail to persist in the host population. But the failure to persist may actually result in the extinction of both the host population and the pathogen itself (Woodroffe 1999). The pathogens which cause serious population declines or severe reductions in host fertility are unlikely to persist in small populations (Lyles & Dobson 1993). Therefore, it is those populations of endangered species which are already reduced to low numbers which may be particularly vulnerable to the introduction of infectious disease from another reservoir host (Grenfell & Dobson 1995, Woodroffe *et al.*, 1997). Indeed, infectious disease has, over the past twenty years or so, become of increasing concern to wildlife conservation (Dobson & Hudson 1986, Scott 1988, Thorne & Williams 1988, Macdonald 1993, Young 1994, Williams & Thorne 1996, Ginsberg 2001) and is considered to be one of the factors in the 'extinction vortex' of endangered species, acting in conjunction with other factors such as habitat loss, drought, and other stochastic factors to drive already small populations to extinction (Caughely & Gunn 1996).

## **1.2 Carnivore conservation and generalist canid pathogens**

Efforts have been made to identify the diseases of potential importance to carnivores. Of 52 diseases of large carnivores, 44% were viral of which many were endemic in carnivores and/or capable of infecting multiple taxonomic families (Murray *et al.*, 1999). The “generalists”, which can infect, and be transmitted by, a wide range of both domestic and wildlife species are those which are most frequently responsible for wildlife epidemics (Cleaveland 2003). Hence it is those pathogens which have broad host ranges, direct and effective modes of transmission (such as inhalation or ingestion), and high mortality rates which are of the greatest conservation concern (Woodroffe 1999, Funk *et al.*, 2001, Cleaveland *et al.*, 2002).

If a small population cannot maintain a generalist pathogen it follows that these pathogens must persist in another reservoir population which meets the CCS requirement (Haydon *et al.*, 2002). The small populations affected by generalist canid pathogens are usually ‘spill-over’ hosts which do not contribute to the maintenance of the pathogen in its primary host species (Woodroffe *et al.*, 2004). The ability of viruses such as rabies and canine distemper to persist in larger host populations of domestic dogs (*Canis familiaris*) has contributed to them becoming important wildlife pathogens. In the Serengeti, for example, declines of the African wild dog (*Lycaon pictus*) population in the 1960s and 1970s were attributed in part to canine distemper or rabies, the latter being responsible for an epidemic during which the few remaining individuals disappeared (Creel & Creel 1998).

The spread of generalist pathogens is augmented by human-driven changes in land use, environment, climate, human demography and the movements of humans and their domestic animals (Daszak *et al.*, 2000). Human encroachment on wildlife areas has resulted in the emergence of a number of human pathogens such as HIV (Hahn *et al.*, 2000) and Ebola (Daszak *et al.*, 2000). But of concern to carnivore conservation

is the increased contact with domestic species, as carnivores are susceptible to a wide array of highly lethal or debilitating microparasites many of which are endemic in domestic species (Murray *et al.*, 1999). Indeed, a study investigating the factors responsible for extinction of carnivores in protected areas found that contact with human activity (and therefore domesticated species) on the edges of protected areas was responsible for the majority of mortalities in protected carnivore populations (Woodroffe & Ginsberg 1998).

The most widespread of the domesticated animals is the domestic dog which has spread, with man, to every continent and most islands (WHO & WSPA 1990, Wandeler *et al.*, 1993). The world population is estimated at 500 million which makes the dog the most abundant of the carnivores (MacPherson *et al.*, 2000). Disease surveys of the large and growing domestic dog populations of developing communities in Africa indicate that canine distemper, rabies, CPV-2 and other viral and macroparasitic infections are highly prevalent or enzootic, largely uncontrolled by the low levels of vaccination and health care available to animals in poor, resource-limited developing communities (Rautenbach *et al.*, 1991, Eckersley *et al.*, 1992, Odendaal 1994, Minnaar *et al.*, 1999, Butler & Bingham 2000, Leisewitz *et al.*, 2001, Minnaar & Krecek 2001, Minnaar *et al.*, 2002). Dogs pose a health threat to both humans and wildlife as they can transmit over 100 zoonoses (WHO & WSPA 1990) and many protected areas, such as the Serengeti National Park, are surrounded by ever growing large domestic dog populations (Cleaveland 1996, Macdonald 1996). Domestic dogs can interact with wild carnivores along the boundaries of reserves (for example, at carcasses) and therefore exposing sympatric wildlife to the diseases they carry (Creel & Creel 1998, Butler & du Toit 2002, Butler *et al.*, 2004). It is therefore the generalist *canid* pathogens which are of most concern to carnivore conservation (Cleaveland *et al.*, 2001, Laurenson *et al.*, 2004, Woodroffe *et al.*, 2004).

Indeed, a growing number of mass mortalities and population declines in terrestrial and aquatic wild carnivores have been attributed to spill-over of infectious disease from domestic dogs (Grachev *et al.*, 1989, Osterhaus *et al.*, 1989, Bengston *et al.*,

1991, Alexander & Appel 1994, Cleaveland & Dye 1995, Haas *et al.*, 1996, Roelke-Parker *et al.*, 1996, Sillerio-Zubiri *et al.*, 1996, Peterson *et al.*, 1998, Cleaveland *et al.*, 2000, Funk *et al.*, 2001). Dogs may come into contact with wildlife where they invade protected areas or where wildlife may leave these areas (Laurenson *et al.*, 2004). However, the exact nature of the contact between dogs and wild carnivores has not been accurately determined for the majority of these outbreaks and it is possible that disease transmission between wild carnivores of conservation interest and domestic dogs does not always occur via direct contact (Macdonald 1996, Laurenson *et al.*, 1998).

Domestic dogs are likely to come into contact with some wild carnivores more than others. Wildlife species that are both common and widespread, such as foxes (*Vulpes* spp.), coyotes (*Canis latrans*) and jackals (*Canis* spp.) are ‘opportunistic generalists’ because they flourish in both human-altered and wildlife landscapes; they are therefore likely to reach the CCS required for the persistence of generalist pathogens (Laurenson *et al.*, 2004). The ecology of wild canid species also facilitates the transmission of infection as they are territorial, social and they may eat infected prey (Creel *et al.*, 1995, Scheepers & Venzke 1995, Laurenson *et al.*, 2004). In addition, most wild canids are closely related to the domestic dog (Xiaoming *et al.*, 2004) and therefore are likely to share its susceptibility to a wide range of generalist pathogens. Therefore, a few key species which exist in a variety of landscapes may act as key links in the chain of disease transmission between domestic dogs and wildlife and their intra- and inter-specific contacts are likely to be important factors in the dynamics of generalist canid pathogens.

Of the 12 species of the *Canidae* found in Africa (Kingdon 1997) the 3 jackal species are the most widespread and abundant. Opportunistic wild canids such as the jackal species are good examples of ‘ecological generalists’ as they do not have any specialized habitat or food requirements and, as discussed in the following section. Furthermore, as they can persist in human dominated landscapes, jackals are ideally placed to act as the connection between the growing domestic dog populations and wildlife. Although studies have implicated the jackal species in the maintenance and

transmission of rabies (Barnard 1979, Bingham & Foggin 1993, Nel *et al.*, 1993, Smith *et al.*, 1993, Swanepoel *et al.*, 1993a, Swanepoel *et al.*, 1993b, Courtin *et al.*, 2000, Hofmeyr *et al.*, 2004), their role in the transmission of other generalist canid pathogens has largely been ignored. The majority of wildlife epidemiology studies have focused on species of conservation concern (e.g. the Ethiopian wolf, *Canis simiensis*) with studies of jackals limited to serological surveys of exposure (Alexander *et al.*, 1994, Spencer *et al.*, 1999, Sharmir *et al.*, 2001). This thesis will address this gap by exploring their role in the epidemiology of 4 generalist canid pathogens.

### **1.3 Investigation of a reservoir species**

Generalist canid pathogens, such as rabies and CDV, pose additional conceptual and practical challenges relative to host-specific pathogens because they possess such broad host ranges: different wild and/or domestic hosts may act as reservoirs under different epidemiological circumstances.

Haydon *et al.* (2002), incorporating the fact that generalist pathogens may be maintained or transmitted by more than one species (and/or a contaminated environment), defined a reservoir as

*“.....one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population.”*

(Haydon *et al.*, 2002)

Haydon *et al.* (2002) went on to propose specific terminology which can be used to describe the complex ‘target-reservoir’ system identified by the above hypothesis.

Wildlife, such as jackals, which are not of conservation interest are termed the *non-target* populations, and are the potentially susceptible host populations which may be connected (directly or indirectly) to the *target* population i.e. the carnivore of



conservation concern. The non-target populations may constitute all or part of the reservoir. The reservoir is a) a *maintenance* population or b) a *maintenance community* composed of one or more (non-) target species, of which the total population size exceeds the critical community size for the persistence of the pathogen (Bartlett 1960); non-maintenance populations are below the critical community size. A population which transmits infection directly to the target population is a *source* population which may or may not be part of the maintenance community. In the case of a pathogen which can persist in the environment, such as canine parvovirus (CPV) (Pollock 1982, Gordon & Angrick 1986, Hoskins 1998), the environment itself may also be part of the maintenance community.

The practical challenge lies in untangling the intricate web of transmission to identify the species, and which populations, are maintaining and which are simply transmitting the virus in the field. To this end, Haydon et al. (2002) put forward a number of 'practical indicators' which can be used to identify reservoirs (Figure 1-3). Not all of these indicators may be necessary to identify a reservoir (an intervention study would provide ultimate confirmation of the existence of a reservoir) but they should be regarded as individual pieces of a jigsaw which may point towards one or more species as potential maintenance hosts.

**Figure 1-1: Practical steps for the identification of a reservoir**

- Evidence of natural infection in both the source and target populations
- Evidence of transmission between source and target populations
- Evidence of persistence of infection in the maintenance population or community
- Evidence of high antigenic or genetic similarities between pathogen isolated from source and target populations
- An intervention study targeting disease in the maintenance population or community must result in a decrease in incidence in the target population

Cases in the (non-target) source and target populations may be associated in time with case numbers in the target population peaking after those in the source population. Such a correlation may be hard to detect, especially if transmission is sporadic. Data may comprise anecdotal accounts or detailed temporal disease patterns e.g. time series analyses of cases of rabies in jackals and dogs (Cleaveland & Dye 1995, Courtin *et al.*, 2000). There must also be evidence of natural infection in the target and suspected maintenance population(s). This can, depending on the

resources available and the longevity of the infection, be evidence of exposure from a serological survey or, preferably, isolation of the antigen or genetic material from the host. A high seroprevalence alone is not sufficient evidence that a population is acting as a reservoir, indeed, pathogens can persist at low prevalences.

Evidence of transmission from non-target source populations to target populations is also useful – for example, the presence of the pathogen's transmission stages in excretory or other bodily substances and/or proof of actual contact, capable of transmitting disease, between individuals in the source and target populations. In many studies, a sympatric distribution alone is assumed to provide sufficient opportunities for disease transmission. Evidence of persistence in the non-target population(s) can only be obtained from longitudinal studies. Longitudinal serological data can give a good indication but ideally, the pathogen would be isolated repeatedly from the same population over a number of years. It may be possible to show that the same pathogen is circulating in the target and source populations using molecular techniques. Recent advances in molecular methodologies have greatly improved the amplification of the pathogen DNA or RNA from tissues and other types of samples (Rypula *et al.*, 2002, Kumar *et al.*, 2003, Decaro *et al.*, 2004) but one must consider that identification of similar strains does not provide evidence of directionality of transmission.

Lastly, the ultimate proof for the existence of a reservoir would involve an intervention study, which when applied to the suspected maintenance host(s), should decrease the incidence of disease in the target species(s) or result in the elimination of the pathogen. This would provide evidence of the presence of a maintenance host(s). The intervention may be a vaccination campaign and/or a form of restriction aimed at reducing contacts between target and non-target species.

This thesis applies the theoretical framework and practical indicators outlined above to investigate the role played by black-backed jackals (*Canis mesomelas*) in the transmission of a number of generalist canid pathogens, reviewed in the next section.



## 1.4 Aspects of the biology and ecology of the jackal affecting the epidemiology of generalist canid pathogens

Of the 12 members of the *Canidae* which occur in Africa 4, including the jackals, belong to the genus *Canis* (Table 1-1) (Kingdon 1997). Jackals are the most abundant and widespread of the larger carnivores in Africa of which the black-backed jackal is the most prevalent in semi-arid sub-Saharan Africa (Kaunda & Skinner 2003).

**Table 1-1: The members of the genus *Canis* which occur in Africa and their distributions in terms of land area.** Total area is an approximate measure of the total area of the African continent occupied by a particular species. Adapted from Kingdon (1997).

Species	Classified by	Common name	Total area (%)
<i>Canis simiensis</i>	Rüppel (1935)	Ethiopian wolf	<1
<i>Canis adustus</i>	Sundevall (1846)	Side-striped jackal	c.33
<i>Canis mesomelas</i>	Schreber (1775)	Black-backed jackal	c.20
<i>Canis aureus</i>	Linnaeus (1758)	Golden jackal	c.40

Of the three jackal species, the golden jackal (*Canis aureus*) occurs in the Sahara and in arid regions of eastern Africa (Kingdon 1997), whereas only the side-striped (*Canis adustus*) and the black-backed jackal occur in southern Africa (Lombaard 1971, Macdonald 2001).

**Figure 1-2: The distribution of the black-backed jackal in sub-Saharan Africa.** (Ginsberg & Macdonald 1990).



As a social canid, there are many basic characteristics, both biological and ecological which make *C. mesomelas* particularly suited to the transmission of generalist canid pathogens. A review by McKenzie (1993) highlighted the aspects of the habitat, home range, territoriality, activity patterns, reproduction, diet and population density which theoretically make the jackal an ideal rabies vector, but the same characteristics may make the jackal an ideal host for all generalist canid pathogens. A brief overview of these characteristics is given below, based on the review by McKenzie (1993) and additional selected references.

*Habitat* The black-backed jackals is highly adaptive and can be found in a wide range of habitats, from upland veld to coastal desert (Kingdon 1997). These jackals tend to favour the drier environments (*C. adustus* predominates in the wetter regions) (Apps 2000) and are well suited to life in the desert (Hiscocks & Perrin 1987, Hiscocks & Perrin 1988, Nel *et al.*, 1997, Henschel 2001) as they are independent of water (Apps 2000). *C. mesomelas* can also be found in dense woodland (McKenzie 1993), peri-urban areas (Apps 2000), montane (Rowe-Rowe 1982) and open grasslands and Savannah (Kingdon 1997, Loveridge & Macdonald 2002). In conclusion, jackals are very adaptable and will thrive in a wide variety of habitats.

*Social system, home ranges, dispersal and territorial defence* Adult black-backed jackals live as monogamous pairs, with one or more un-paired or immature offspring which stay on to help rear cubs (Moehlman 1979, Moehlman 1983). Both males and females of mated pairs will actively defend the shared territory and aggressive interactions do occur. Members of a pair will scent mark with urine and faeces and vocalise to delineate the territorial boundaries (Moehlman 1983, Apps 2000). Young jackals are independent of the den at 14 weeks will move over larger distances than adults and disperse at about 1-2 years of age; jackals can disperse over distances of more than 100km (Bothma, 1971, cited in McKenzie, 1993). Home ranges of immature and un-paired jackals (those under 3 years of age) overlap considerably with those of adults which tolerate younger individuals so long as they show signs of submission (Ferguson *et al.*, 1983). Adult jackal home ranges do not normally overlap and vary considerably in size depending on the ecological conditions e.g.

habitat type, and resource availability (Loveridge & Nel 2004). In conclusion, the black-backed jackal is a social canid capable of travelling and dispersing over large distances and these are factors which will promote disease transmission both within and between jackal populations.

*Reproduction and life expectancy* Jackals give birth to an average of 5 cubs (range 2-8) after a 2 month gestation period but only 1-2 of these survive to 14 weeks (Apps 2000). Jackals suffer from predation by hyenas, leopards (*Panthera pardus*) (Loveridge & Nel 2004) and also brown hyenas (*Hyaena brunnea*) on the coast in Namibia (pers. comm. R. Braby, Ministry of Environment and Tourism; N. Jenner, IOZ Jackal Project). Cubs are raised on milk and regurgitated food and are weaned at 8-10 weeks of age and begin foraging at 14 weeks (Apps 2000). Cubs venture away from the den at 3 months and remain in the natural home range for at least 6 months (Ferguson *et al.*, 1983). Individuals are sexually mature at 11 months and fully grown at just over a year but they will only breed when they have formed a monogamous pair bond, which is usually life-long, and established a territory, often at two to three years of age (Moehlman 1983, Apps 2000). Jackals may live up to 10-12 years of age in the wild but few are thought to live beyond 7 years (Lombaard 1971, Loveridge & Nel 2004). In conclusion, the black-backed jackal has both a good reproductive potential and a reasonably long lifespan, characteristics which make it resilient, allowing persistence in the face of disease and human persecution. The black-backed jackal is unlikely to suffer serious long-term population declines or extinction.

*Diet* Black-backed jackals are highly flexible in that they are omnivorous and opportunistic and they are both predators and scavengers (Kingdon 1997, Macdonald 2001, Atkinson *et al.*, 2002, Loveridge & Macdonald 2003, Admasu *et al.*, 2004). Large groups of jackals will congregate at carcasses (Macdonald 2001) where they may come into contact with other scavengers such as hyenas and domestic dogs (Admasu *et al.*, 2004, Loveridge & Nel 2004). They will feed on invertebrates, fruits, birds, small mammals, and carrion but their small size precludes the hunting of large prey and they often lose prey to other scavengers (Lamprecht 1978). The jackals'

diet reflects the variety of accessible food items available in the habitat and they will make use of human-derived resources such as rubbish (Kok 1996). Black-backed jackals on the Namibian coast have a much more restricted diet, dominated by seals and birds (Stuart 1976, Hiscocks & Perrin 1987, Nel *et al.*, 1997, Oosthuizen *et al.*, 1997b). In conclusion, black-backed jackals have a flexible diet allowing them to adapt to a wide range of habitats. The sharing carcasses and a range of habitats will bring them into contact with a wide range of species.

*Population density* Like home range, population densities vary considerably both seasonally and between habitats. The population density of black-backed jackals at Hwange nature reserve (Zimbabwe) ranged between 0.5-0.8/km<sup>2</sup> to 0.7-1.0/km<sup>2</sup> after pups were born (Loveridge & Macdonald 2002). A density of 1/km<sup>2</sup> was recorded in commercial farmland in Zimbabwe where large predators are absent (Bingham & Foggin 1993). Jackals can also reach very high densities, c.22/km<sup>2</sup>, at clumped resources such as the Cape fur seal (*Arctocephalus pusillus pusillus*) colonies on the Namibian coast (Hiscocks & Perrin 1988). Jackals may therefore reach sufficient densities and attain the CCSs necessary for the persistence of range of canid pathogens.

In summary, 'flexibility' is the word which best describes the black-backed jackal. As a resilient predator and scavenger, persisting in both human and wildlife habitats it is ideally placed to act as a transmission route between domestic and wildlife species. Its reproductive potential and resilience mean that it is likely to recover quickly from disease outbreaks and that it may reach the CCSs to allow it to be a reservoir for many canid pathogens.

However, despite the adaptability and resilience of the jackal, it is not possible to make generalisations regarding the role played by jackals in the maintenance and transmission of generalist canid pathogens because their exact role will depend on the particular ecological circumstances and pathogen characteristics – the introduction of the same disease into different jackal populations in different habitats, or the introduction of a different disease in the same ecological

circumstances may have very different consequences. Understanding how, and then being able to predict whether, jackals are acting as a disease reservoir in a particular situation will greatly enhance any disease control and conservation efforts in sub-Saharan Africa. If jackals are a reservoir for virulent canid pathogens, control of the diseases in the dog population for the protection of endangered species, will have limited success (Appel 1987b, Chappuis 1995). It is therefore essential to correctly identify the reservoir for a particular pathogen in the particular carnivore community of interest as different host species may be reservoirs in different ecological and epidemiological circumstances (Laurenson *et al.*, 2004).

#### **1.4.1 Exposure of jackals to canid pathogens**

Free-living black-backed jackals in a number of African countries have been found to be exposed to a wide range of pathogens (Table 1-2). In addition, a number of experimental infections have confirmed the susceptibility of this species to rickettsial and helminth parasites (Table 1-2). Although these studies do not shed light on the epidemiological role of the jackals, they do indicate which pathogen(s) may be circulating in a carnivore community or which pathogen(s) are a cause for concern. They may also indicate, by taking account of their susceptibility to different diseases, that jackals could be a useful indicator species for monitoring the prevalence of specific canid diseases, thus helping assess exposure to the species of concern (Alexander *et al.*, 1994).

**Table 1-2: The exposure of black-backed jackals to infection with a range of viral and macroparasite canid infections. 'ET' experimental transmission; <sup>a</sup>Confirmed infections.**

Pathogen	Country	Percentage of individuals which resulted seropositive or infected
Rabies <sup>12,13</sup>	Namibia	26.1% (1975-1990)
	South Africa	4.8% (4,754 <sup>a</sup> )
	Botswana	69.2% (279 <sup>a</sup> )
	Zimbabwe	79.8% (1,999 <sup>a</sup> )
Canine distemper <sup>1,3</sup>	Kenya	9.0% (55)
	Zimbabwe	63.6% (14)
Canine parvovirus <sup>1,3</sup>	Kenya	25.5% ( 55 )
	Zimbabwe	18.2% ( 22 )
Canine adenovirus type-1 <sup>3</sup>	Zimbabwe	9.1% ( 22 )
Sarcoptic mange <sup>14,11</sup>	Namibia	-
	South Africa	-
<i>Ehrlichia canis</i> <sup>1,2,4</sup>	Kenya	2.6% ( 39 )
	Israel	54.3 ( 46 )
	South Africa	ET ( 4 )
<i>Babesia canis</i> <sup>4</sup>	South Africa	ET (4)
<i>Leishmania donovani</i> <sup>5</sup>	South Africa	-
<i>Toxoplasma gondii</i> <sup>5</sup>	South Africa	-
<i>Ancylostoma caninum</i> <sup>6</sup>	Somalia	-
<i>A. braziliense</i> <sup>6</sup>		
<i>A. somaliense</i> <sup>6</sup>		
<i>Echinococcus granulosus</i> <sup>7,8,9</sup>	Kenya	28.9% ( 38 )
		38.5% (13)
	South Africa	9.7%
<i>Spirocerca lupi</i> <sup>10</sup>	Namibia	-

References: 1 Alexander *et al.*, 1994; 2. Sharmir *et al.*, 2001; 3 Spencer *et al.*, 1999; 4 Van Heerden (Van Heerden 1980); 5 Van der Merwe (Van der Merwe 1953); 6 Gupta *et al.*, 1988 cited in Macchioni (Macchioni 1995); 7 MacPherson *et al.* (MacPherson *et al.*, 1983); 8 Eugster 1978 cited in Macpherson *et al.*, 1983; 9 Verster & Collins (Verster & Collins 1966); 10 Pers. comm. Dr. F. Mettler, Veterinary pathologist, CVL, Namibia; 11 Zumpt & Ledger 1973; 12 Berry 1993; 13 Swanepoel *et al.*, 1993a; 14 Schneider 1994.

## 1.5 Generalist canid pathogens investigated in this study

Rabies is known to affect black-backed jackals throughout southern Africa (Swanepoel *et al.*, 1993b) and to have caused serious population declines in endangered species such as the Ethiopian wolf and the African wild dog (Alexander *et al.*, 1993b, Gascoyne *et al.*, 1993, Sillerio-Zubiri *et al.*, 1996, Randall *et al.*, 2004). Rabies is not, however, the only generalist canid pathogen of concern to carnivore

conservation (Murray *et al.*, 1999). This study focuses on 4 other canid pathogens, namely canine distemper virus (CDV), canine parvovirus type-2 (CPV-2), canine adenovirus type-1 (CAV-1) and sarcoptic mange. Particular emphasis is placed on CDV due to its high-profile (re-)emergence in wildlife populations, its apparent ability to infect new species, and the resulting mass-mortalities (Harder & Osterhaus 1997, Cleaveland 2003). A review of the basic characteristics of CDV, CPV-2, CAV-1 and sarcoptic mange is given in the following section.

### **1.5.1 Canine distemper**

#### *1.5.1.1 Terrestrial carnivores*

Canine distemper is an acute or sub-acute highly contagious disease that can affect the respiratory, gastro-intestinal or central nervous systems. CDV is a member of the *Paramyxoviridae* of the genus *Morbillivirus* which is well known for serious diseases of both humans (measles) and animals (rinderpest). CDV is a relatively large single-stranded RNA virus (Appel 1987b) and a true generalist as infection has been reported in many different species of 8 of the 11 families of the order *Carnivora* (Montali *et al.*, 1987). CDV occurs worldwide and is the most common neurological infection in dogs (Zurbriggen & Vandewelde 1994). Exposure to, or infection with, CDV has been reported for a wide range of canid species in Africa and elsewhere (Table 1-3) and all members of the *Canidae* are susceptible to infection with CDV (Appel 1987b).



**Table 1-3: Suspected and confirmed infection or exposure to CDV in free-living and captive canid species.**

Species	Area	Evidence for exposure and/or infection	Reference
African wild dogs ( <i>Lycaon pictus</i> )	Bushmanland, Namibia	Serological survey	(Laurenson <i>et al.</i> , 1997b)
	Masai-Mara, Kenya	Serological survey	(Alexander <i>et al.</i> , 1993a)
	Mkomazi, Tanzania	Confirmed mortality	(van de Bildt <i>et al.</i> , 2002)
Australian dingoes ( <i>Canis dingo</i> )	Zoological park, Australia	-	(Deem <i>et al.</i> , 2000)
Maned wolves ( <i>Chrysocyon brachyurus</i> )	Brazilian zoos	Serological survey	(Maia & Gouveia 2001)
Bat-eared foxes ( <i>Octocyon megalotis</i> )	Serengeti, Tanzania	Confirmed mortality	(Roelke-Parker <i>et al.</i> , 1996)
Swift foxes ( <i>Vulpes velox</i> )	Western USA	Serological survey	(Miller <i>et al.</i> , 2000)
Kit foxes ( <i>Vulpes macrotis</i> )	Western USA	Serological survey	(Miller <i>et al.</i> , 2000)
Racoon dogs ( <i>Nyctereutes procyonoides</i> )	Tokyo, Japan	Histopathology, immunocytochemistry	(Machida 1993)
Coyotes ( <i>Canis latrans</i> )	Utah, California, USA	Serological survey	(Cypher <i>et al.</i> , 1998, Arjo <i>et al.</i> , 2003)
Red foxes ( <i>Vulpes vulpes</i> )	Georgia and other states, USA	Histopathology	(Little <i>et al.</i> , 1998b)
Gray foxes ( <i>Urocyon cinereoargenteus</i> )	Sarasota, Florida, USA	Histopathology, infection studies of field isolates	(Hoff & Bigler 1974)
Wolves ( <i>Canis lupus</i> )	Northwest Territories, Canada	Serological survey	(Choquette & Kuyt 1974)
Jackals ( <i>Canis mesomelas</i> , <i>C. aureus</i> , <i>C. adustus</i> )	Masai-Mara, Kenya	Serological survey	(Alexander <i>et al.</i> , 1994)
	Israel		(Sharmir <i>et al.</i> , 2001)
	Zimbabwe		(Spencer <i>et al.</i> , 1999)
Ethiopian wolves ( <i>Canis simensis</i> )	Bale region, Ethiopia	Serological survey	(Laurenson <i>et al.</i> , 1998)



In dogs, the duration and severity of clinical disease vary greatly with viral strain, ranging from asymptomatic infections to acute disease with a mortality rate of approximately 50% (Appel 1987b). Mortality in wildlife also varies considerably, approaching 100% in domestic ferrets (*Mustela putorius furo*) (Montali *et al.*, 1987) and 30% in African lions (*Panthera leo*) (Roelke-Parker *et al.*, 1996). CDV has been responsible for serious population declines of terrestrial carnivores including black-footed ferrets (*Mustela nigripes*) (Williams *et al.*, 1988), African wild dogs (Alexander *et al.*, 1996, van de Bildt *et al.*, 2002), and African lions (Roelke-Parker *et al.*, 1996, Kock *et al.*, 1998).

Insights into the problems associated with, and the effects of, the establishment of CDV in an abundant wildlife species can be obtained by considering the epidemiology of distemper in raccoons (*Procyon lotor*) in North America. Raccoons, like jackals, are successful and resilient omnivores with high reproductive rates and are adaptable to most habitats (Rosatte 2000). Raccoons abound in both wildlife and urban environments in which they can achieve extremely high densities, exceeding 100 individuals per km<sup>2</sup> in some areas (Rosatte 2000, Smith & Engeman 2002), with frequent interactions between humans and wildlife (Hoff & Bigler 1974). Urban, suburban and rural populations are frequently subject to rabies and CD epidemics and raccoons are thought to be a reservoir for both these pathogens (Hoff & Bigler 1974, Roscoe 1993, Riley *et al.*, 1998, Rosatte 2000). Raccoons are thought to be the source of CDV which affected captive carnivores in zoos and reserves (Appel *et al.*, 1994) and to be responsible for the spread of CDV between counties (Hoff & Bigler 1974).

#### *CDV infection in dogs*

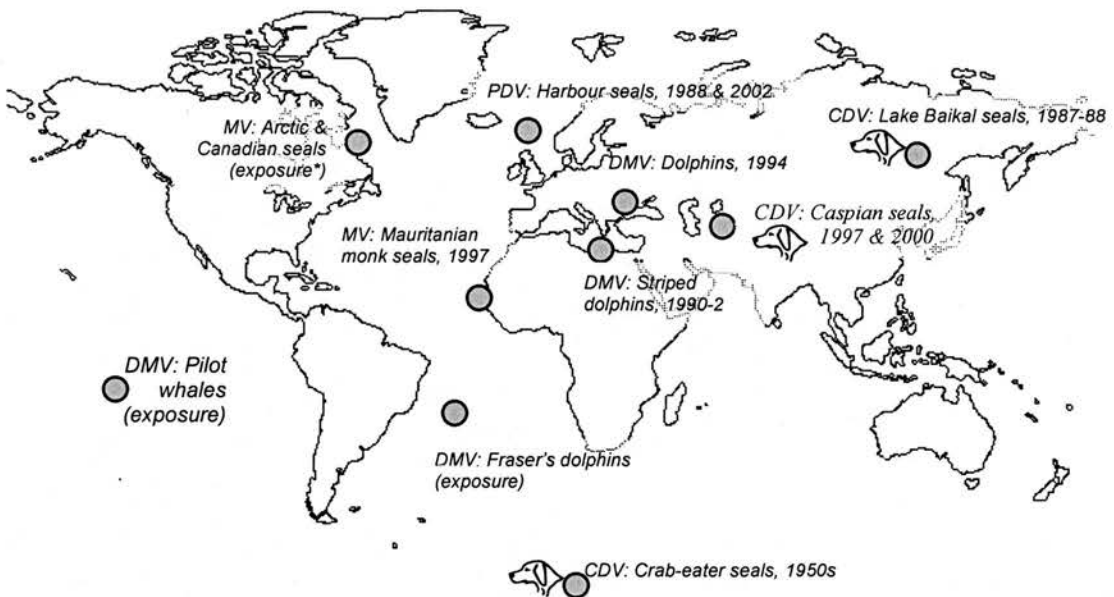
Natural CDV infection in domestic dogs has been well characterised (Greene & Appel 1998). CDV is transmitted by oronasal exposure to aerosolised body fluids, mainly respiratory secretions (Gorham 1966) but it can be found in all body secretions during acute infections, including urine regardless of whether or not the dog becomes symptomatic (Appel 1987b). CDV is not very environmentally resistant

but it can persist in tissues for hours at room temperature and for weeks at cold temperatures (Greene & Appel 1998). Once in the upper respiratory tract it spreads to lymph nodes and lymphoid organs and by 8 to 9 days post-infection it has spread to the CNS (central nervous system) tissues and all epithelial tissues - this spread is associated with the end of the latent period and the start of virus secretion (Greene & Appel 1998). At this point, dogs with an adequate immune response, characterised by a high virus neutralising (VN) antibody titre (well over 1:100 (Appel 1970)), clear the infection, usually with no clinical signs; indeed, 50-70% of domestic dogs may be asymptotically infected (Greene & Appel 1998). The severity of the disease is inversely related to the VN antibody titre so that individuals which produce no antibody response go on to develop severe multi-systemic illness which is soon followed by death (Appel 1969). Those which develop a low (likely a little over a titre of 1:100) antibody response will usually develop mild or unapparent illness but can go on to shed virus for up to 60 days (Appel 1987b, Greene & Appel 1998). Neurological symptoms due to virus infection of the CNS may occur during the acute phase of the disease or weeks or months later (Appel & Summers 1995). The humoral immune response in recovered dogs is thought to be very durable and likely lifelong as recovered individuals are resistant to re-infection for 7 years (Appel 1987b).

#### 1.5.1.2 *Marine species*

CDV has recently expanded its host range to include members of the *Pinnipedia* (Harder & Osterhaus 1997, Baumgartner *et al.*, 2003). A factor in this increased host range is likely to have been the growth of the human population and the subsequent encroachment of domestic dogs into wildlife areas, resulting in increased opportunities for cross-species transmission. The introduction of this virus, thought to have occurred from domestic dogs, into naïve pinniped populations has caused mass mortalities in Lake Baikal seals (*Phoca siberica*) (Grachev *et al.*, 1989, Mamaev *et al.*, 1995), Caspian seals (*Phoca caspica*) (Kennedy *et al.*, 2000) and crab-eater seals (*Lobodon carcinophagus*) (Bengston *et al.*, 1991, Barrett 1999) (Figure 1-3).

**Figure 1-3: Examples of the exposure to, and mass mortalities caused by, morbilliviruses in different regions of the world. \*Exposure in harbour, grey, harp and hooded seals. Suspected spill-over from domestic dogs indicated by**



References: (Grachev *et al.*, 1989, Bengston *et al.*, 1991, Henderson *et al.*, 1992, Ross *et al.*, 1992, Mamaev *et al.*, 1996, Forsyth *et al.*, 1998, Birkun *et al.*, 1999, van de Bildt *et al.*, 1999, Kennedy *et al.*, 2000, Van Bresseem *et al.*, 2001).

Morbillivirus infections in marine mammals were first discovered in 1988 (Osterhaus & Vedder 1988) when a mass mortality occurred in European harbour (*Phoca vitulina*) and grey seal (*Halichoerus grypus*) populations of the North Sea (Figure 1-3). The virus responsible for the 1988 mass mortality was later identified as phocine distemper virus (PDV) (Mahy *et al.*, 1988), a new member of the morbillivirus genus thought to have evolved from CDV (Barrett 1999) and also responsible for the mortality in harbour seals in the North Sea in 2002 (Jensen *et al.*, 2002). PDV is not restricted to marine species as it caused fatal disease in mink (Blixenkrone-Moller *et al.*, 1990). There is now strong virological, pathological, epidemiological and experimental evidence that morbilliviruses can fatally infect seals (Barrett *et al.*, 1995).

The spread of CDV to pinnipeds is thought to have been a recent event but morbilliviruses may have been present in pinniped populations long before the 1988

outbreak, or the detection of exposure to CDV. Archival sera from Canadian seals were reactive with both CDV (Ross *et al.*, 1992) and PDV (Henderson *et al.*, 1992) and these seals were sampled prior to the 1988 European outbreak. Hence pinniped populations may have been previously exposed to morbilliviruses without experiencing an increase in disease-related mortality. Prior exposure to CDV without an increase in disease-related mortality has also been detected in felids (Roelke-Parker *et al.*, 1996).

Since the 1988 mass mortality other morbilliviruses, namely the porpoise morbillivirus (PMV) and the dolphin morbillivirus (DMV) have been isolated from harbour porpoises (*Phocoena phocoena*) (Kennedy *et al.*, 1988) and striped dolphins (*Stenella coeruleoalba*) in the Mediterranean (Domingo *et al.*, 1990) respectively, and from a variety of marine mammals in the Atlantic (Barrett *et al.*, 1995, Duignan *et al.*, 1995, Van Bressem *et al.*, 2001). Hence the morbillivirus genus has been expanded to include PMV and DMV which are distinct from PDV and more closely related to ruminant morbilliviruses than to CDV and PDV (Visser *et al.*, 1993a). Exposure to morbilliviruses has been detected in a wide range of marine species throughout the world including North American harbour, grey, harp (*Pagophilus groenlandia*) and hooded seals (*Cystophora cristata*) (Henderson *et al.*, 1992, Ross *et al.*, 1992), monk seals (*Monachus monachus*) off the coast of Mauritania, (Figure 1-3) and dolphins and whales in the Atlantic and Pacific oceans respectively (Van Bressem *et al.*, 2001).

### **1.5.2 Canine parvovirus types 2a and 2b**

The canine parvoviruses belong to the Parvovirus genus of the *Parvoviridae* family (DNA viruses) which includes a number of other parvoviruses, such as the feline parvovirus (FPV) and the mink enteritis virus (MEV), which infect carnivores. Canine parvovirus type 1 (CPV-1), isolated in the late 1960s, is known to affect only dogs (Carmichael 1987). CPV-2 emerged as a fatal enteric disease of dogs in the late 1970s and was subsequently replaced by two antigenic variants CPV-2a and CPV-2b which now coexist in dog populations worldwide (Parish 1994, Truyen & Parish

1995). There are a number of hypotheses to explain the sudden appearance and rapid spread of CPV-2, including the idea that CPV-2 may have emerged from a FPV-like virus in a wild carnivore and later adapted to infect canids (Parish 1994, Truyen & Parish 1995). The most striking change in the evolution of CPV-2 and its antigenic types is the loss and recovery of the felid host range (Truyen & Parish 1995). CPV-2 was not capable of infecting felids but its antigenic variants, CPV-2a and 2b, are capable of infecting both the smaller domestic cats and the larger wild felids (Truyen 1996, Steinel *et al.*, 1998, Munson *et al.*, 2000, Ikeda *et al.*, 2002). CPV-2a and 2b therefore, not only have a worldwide distribution but are also capable of infecting wild and domestic carnivores. Exposure to, or infection with, CPV-2 (from here on in CPV-2 refers to both antigenic variants unless otherwise stated) has been reported in a wide range of wildlife species (Barker & Parrish 2001) including jackals (Alexander *et al.*, 1994, Spencer *et al.*, 1999), wolves (*Canis lupus*) (Cypher *et al.*, 1998, Battilani *et al.*, 2001), coyotes (Arjo *et al.*, 2003), kit foxes (*Vulpes macrotis*) (Cypher & Frost 1999), Canada lynx (Biek *et al.*, 2002) and bears (*Ursus americanus floridanus*) (Dunbar *et al.*, 1998).

CPV-2, like all parvoviruses, is extremely resistant to environmental degradation and may persist in faeces for years or in the environment for months ( $\geq 6$  months at 4°C) at a time (Appel & Parrish 1987). Its ability to persist in the environment for long periods of time greatly increases the chances of its establishment as an endemic infection as the environment may act as a long-lasting source of infection. Although CPV-2 can cause significant mortality in naïve individuals of all ages, once endemic mortality is only normally seen in young dogs, because older dogs are immune (Barker & Parrish 2001). In domestic dogs, infection does not necessarily result in apparent disease as most do not develop overt clinical signs (Appel & Parrish 1987). It is the growing pups which are most likely to suffer severely from infection, because CPV-2 requires rapidly dividing cells for replication and pups lack protective immunity (Appel & Parrish 1987, Hoskins 1998). The high susceptibility of young animals is of concern to carnivore conservation as a reduction in the recruitment, as seen in African wild dogs and gray wolves (*Canis lupus*) (Mech &

Goyal 1995, Creel *et al.*, 1997, Creel & Creel 1998), of a small endangered population may threaten its persistence.

### *CPV-2 infection in dogs*

CPV-2 is highly contagious and infection usually occurs via oronasal exposure to contaminated faeces (Hoskins 1998); acutely infected dogs shed large amounts of virus in the faeces (Appel & Parrish 1987). After infection the virus replicates rapidly resulting in a viraemia after a short incubation period of 4-6 days (Hoskins 1998). CPV-2 can localise in the epithelium of the gastrointestinal (GI) tract, the lymphoid tissues, bone marrow or in the lungs and liver but the main targets are the intestinal epithelium and the myocardium of the heart (Appel & Parrish 1987, Hoskins 1998). In *utero* or neonatal exposure (at less than 8 weeks of age) to CPV-2 may result in myocarditis and death just before or soon after birth (Parish 1994). In pups older than 8 weeks infection results in enteritis and diarrhoea after which secondary infections usually result in death; but mild cases usually recover and develop durable immunity (Hoskins 1998).

### **1.5.3 Canine adenovirus type-1**

There are a wide range of host-specific adenoviruses which infect humans and a range of domestic and wild species (Woods 2001). The canine adenoviruses, namely CAV-1 and CAV-2 are antigenically related DNA viruses (Ford & Vaden 1998, Woods 2001). CAV-2 causes infectious tracheobronchitis and can be the primary pathogen involved in 'kennel cough', which is one of the most prevalent respiratory infections in dogs (Ford & Vaden 1998). Infection with CAV-1, on the other hand, causes infectious canine hepatitis and results in a more distinct pathology.

Since the description of CAV-1 in dogs in the 1930s and 1940s, exposure and infection with this virus have been reported throughout the world in members of the *Mustelidae*, *Canidae*, and *Ursidae* including mink, wolves (Choquette & Kuyt 1974, Zarnke & Ballard 1987), foxes (Truyen *et al.*, 1998, Riley *et al.*, 2004), coyotes



(Cypher *et al.*, 1998, Arjo *et al.*, 2003), African wild dogs (Laurenson *et al.*, 1997b), jackals (Alexander *et al.*, 1994, Spencer *et al.*, 1999), Ethiopian wolves (Laurenson *et al.*, 1998), striped skunks (*Mephitis mephitis*), racoons (Woods 2001), black bears (*Ursus americanus*) (Pursell *et al.*, 1983, Dunbar *et al.*, 1998) and grizzly bears (*Ursus arctos horribilis*) (Zarnke & Evans 1989, Chomel *et al.*, 1998). Transmission between species, namely foxes, dogs and bears has been documented (Cabasso 1981, Pursell *et al.*, 1983) and the high incidence of naturally occurring neutralising antibodies suggests widespread sub-clinical infection (Greene 1998) but some species, such as dogs, red foxes (*Vulpes vulpes*), coyotes and wolves are the most susceptible to infection (Rubarth, S., 1947, cited in Appel 1987a). Although not as durable as CPV-2, CAV-1 can persist for weeks under cool conditions (Appel 1987a) and is highly contagious. CAV-1 is not airborne but can be spread by direct contact, ingestion of urine, faeces or saliva from infected animals (Appel 1987a) or from a contaminated environment; arthropods such as ticks may also transmit the virus (Greene 1998).

#### *CAV-1 infection in dogs*

CAV-1 is most frequently seen in dogs of less than one year of age. A viraemia develops between 4-8 days post infection and the virus spreads to the eyes, kidneys, endothelial tissues (e.g. brain, lungs) and liver, the latter being the main site of viral damage and pathology (Greene 1998). The virus is also secreted in the saliva, urine and faeces. Individuals with a good humoral immune response 7 days post infection will clear the virus and limit the damage to the liver; some of these individuals may develop chronic hepatitis (Greene 1998). Those with a low antibody response may either develop widespread hepatic necrosis which is usually fatal, or acute hepatitis with limited damage which allows recovery; immunity is likely lifelong (Greene 1998). The virus can persist in the kidneys of recovered individuals and may be excreted in urine for 6-9 months post infection (Greene 1998). Dogs which recover often develop a transient 'blue-eye' as a result of corneal oedema and uveitis (Appel 1987a). Care must be taken with the diagnosis of infection from clinical signs since individuals may suffer damage to the CNS resulting in seizures, ataxia depression,

disorientation and coma and these are signs which may also occur in dogs infected with CDV (Greene & Appel 1998).

#### 1.5.4 Sarcoptic mange

Sarcoptic mange is a highly contagious and debilitating skin disease of mammals caused by the mite *Sarcoptes scabiei*, one of three mange mites known to cause disease in domestic and wild carnivores; *Otodectes cynotis* and *Notoedres cati* cause otodectic and notoedric mange respectively. Different types of *S. scabiei* are not designated as separate species but are termed varieties on the basis of morphological variations and named after the host from which they are isolated. Mange in dogs and foxes is caused by *Sarcoptes scabiei* var. *canis* and var. *vulpes* respectively (Bornstein *et al.*, 2001). Although highly host-specific, varieties of *S. scabiei*, otodectic and notoedric mange, can spill-over to wildlife species and are therefore of concern to carnivore conservation. The Arctic fox (*Alopex lagopus semenovi*) suffered increased pup mortality and a population crash due to otodectic mange from domestic dogs (Goltsman *et al.*, 1996). Coatis (*Nasua narica*), pampas foxes (*Pseudalopex gymnocercus*) and panthers (*Felis concolor coryii*) have suffered infection with notoedric mange (Maehr *et al.*, 1995, Valenzuela *et al.*, 2000, Deem *et al.*, 2002) and a high incidence of mange in domestic dogs on the borders of protected areas is considered a threat to regional wild carnivores (Fiorello *et al.*, 2004).

Sarcoptic mange has a worldwide distribution (Muller *et al.*, 2001) and infection has been reported in over 100 species of mammals and marsupials (Bornstein *et al.*, 2001). The epidemiology of sarcoptic mange in wildlife is not well understood and very variable. The effect of sarcoptic mange on wild canid populations, for example, varies considerably. Severe population declines caused by *S. scabiei* have been observed in red foxes (Lindstorm & Morner 1985, Wilkinson & Smith 2001) but coyotes suffer only compensatory mortality (Pence & Windberg 1994). Diagnosis of the infection in wildlife and domestic dogs is laborious, necessitating

histopathological examinations and the isolation of the mite from skin scrapes and biopsies which are notoriously unreliable (Bornstein & Zakrisson 1994).

The duration and mechanism of immunity against sarcoptic mange is also not well understood. There appears to be some immunity in experimentally infected domestic dogs (Arlian *et al.*, 1994, Arlian *et al.*, 1996b) but naturally infected dogs and experimentally infected red foxes do not appear to develop any protective immunity (Little *et al.*, 1998a, Bornstein *et al.*, 2001). It is thought that immunity is dependent on the cellular immune response, rather than the humoral antibody one, with the involvement of Langerhans and other white blood cell types in the epidermis and dermis (Arlian *et al.*, 1994).

#### *Sarcoptic mange infection in dogs*

All *S. scabiei* go through egg, larval and nymph stages in the epidermis of the skin in a life cycle lasting approximately 28 days from mating to adult nymph (Arlian & Vyszenski-Moher 1988b). Mites burrow into the skin, down to the stratum spinosum and feed on live cells and tissue fluid whilst the continuous growth in the skin layers means that the burrows, containing eggs and feces are pushed up and found in the stratum corneum (Bornstein *et al.*, 2001). Mites can be found moving on the surface or within burrows in the skin, which may hold thousands of mites per cm<sup>2</sup> (Arlian & Vyszenski-Moher 1988a), and these may fall off the host. Mange can therefore be transmitted directly or indirectly as mites will survive in the environment for days or weeks, depending on lower temperatures and high humidity for survival (Bornstein *et al.*, 2001).

## 1.6 Aims

The purpose of this study is to determine what role the black-backed jackal plays in the transmission and maintenance of CDV and other generalist canid pathogens, namely CAV-1, CPV-2 and sarcoptic mange. Because these generalist canid pathogens have broad host ranges, the investigations of their dynamics in complex multi-host ecosystems are conceptually and logistically more complex than similar studies of pathogens with narrower host ranges or single host pathogens. Firstly, diagnostic samples must be obtained from a number of potential host species, some of which may be difficult to capture because they occur at very low densities or are simply trap-wary. Secondly, it is necessary to investigate the occurrence of interactions between and within populations as contacts between individuals (or a contaminated environment) will determine the opportunities for disease transmission and therefore influence disease dynamics. The investigation of contacts between individuals is central to our understanding of disease dynamics (Anderson & May 1991) but uncovering these networks of interactions in a multi-host system may take “a lifetime” (Funk *et al.*, 2001). However, the selection of an appropriate study system can greatly facilitate the study of wildlife disease dynamics and yield valuable insights into the roles played by the different host species in the transmission and maintenance of generalist canid pathogens. Such a system would include the source and target species of interest and occur in an environment which would facilitate both the sampling of target and source species as well as the observations of intra- and inter-specific interactions.

The Namibian desert coast was selected as a study system due to its concentrated and localised carnivore populations. The Namibian coastal carnivore guild, described in detail in Chapter 2, comprises black-backed jackals, domestic dogs, brown hyenas and Cape fur seals. The seal colonies scattered along the length of the Namibian coast, support large and concentrated black-backed jackal populations which also come into contact with domestic dogs from neighbouring urban settlements. These guilds therefore not only allow the investigation of the transmission of generalist canid pathogens between terrestrial carnivores but also between pinnipeds and

terrestrial carnivores, also of interest due to the occurrence of morbilliviral epidemics in pinniped populations (section 1.5.1.2).

The aims of this study are as follows:

1. To describe the seroprevalence patterns of CDV, CAV-1, CPV-2 and sarcoptic mange in sympatric and adjacent jackal and dog populations.
2. To determine the cause(s) of disease and to provide a description of disease outbreaks in the jackal, dog and seal populations.
3. To determine if the Namibian Cape fur seal population has been exposed to CDV and the prevalence of exposure.
4. To provide evidence for intra- and inter-specific contacts for the transmission of CDV in the coastal carnivore guild.

Specific hypotheses are formulated using the framework proposed by Haydon *et al.*, (2002) to address the above questions in separate chapters. Chapter 3 focuses on CDV in the terrestrial carnivores i.e. jackals and dogs; Chapter 4 investigates the exposure of the seal population to CDV and other morbilliviruses; Chapter 5 focuses on the exposure of jackals and dogs to CAV-1, CPV-2 and sarcoptic mange; Chapter 6 considers the inter- and intra-specific contacts for the transmission of CDV. The implications of the jackal's role in the transmission and maintenance of generalist canid pathogens in the coastal guild and elsewhere in Namibia, as well as future research, are discussed in Chapter 7.

## **Chapter 2: Materials and Methods**



## **2.1 Abstract**

Investigations of the role played by jackals in the transmission of generalist canid pathogens are hampered by the logistical constraints associated with studying the dynamics of multi-host pathogens, which may persist in a number of different host species and can be transmitted via a complex network of species interactions. This study on the transmission of generalist canid pathogens between black-backed jackals, domestic dogs and Cape fur seals, was based on the Namibian coast, where the occurrence of a limited number of carnivore species and the presence of clumped resources (seal colonies) facilitated data collection and sampling. The study sites were based at the two largest Cape fur seal colonies and the neighbouring towns, and the jackal and dog populations at these sites were sampled yearly between 2001 and 2003. A total of 90 jackals were sampled for serum of which 85 were from captures ( $n=87$ ) and 5 from animals euthanased in the wild due to severe CDV infection. Of 100 dogs included in this study, 92 were sampled for serum from the towns of Swakopmund, Walvis Bay and Lüderitz. Data on condition, age, sex and clinical signs were collected for both species. Tissues sampled from jackals ( $n=10$ ) and dogs ( $n=4$ ) were submitted for the histopathological diagnosis of CDV infection. At the main study site, captured jackals were individually marked and released for the mark and re-sight estimation of population size ( $n=160$ , 95% CI 129-205); the total coastal population was conservatively estimated at *c.*500 individuals. Using human:dog ratios of urban and rural areas, the dog populations of the coastal towns and Namibia were estimated at *c.*13,500 and *c.*400,000 individuals respectively.

## **2.2 Introduction**

This chapter describes the methodologies and data relevant to a number of chapters. Methods and data specific to particular chapters are included in the relevant chapters. Detailed descriptions of the Namibian coast and the study areas are given together with the reasons for the selection of the particular study sites used in this project. This chapter also describes the sampling of the domestic dogs and jackals, with detailed descriptions of the sources of domestic dogs and the capture methods used for jackals. Additional data was also collected by personal communication from a number of sources including veterinarians, Ministry staff and research scientists working on the coast. Separate results and discussion sections are presented for jackals and dogs where some of the biases in the data collection are discussed. How these biases may affect the conclusions and data analyses are discussed in further detail in the specific chapters. Mark and re-sight data and human:dog ratios are used to calculate estimates of the jackal and dog populations respectively and these estimates are used throughout the thesis.

## **2.3 Study areas**

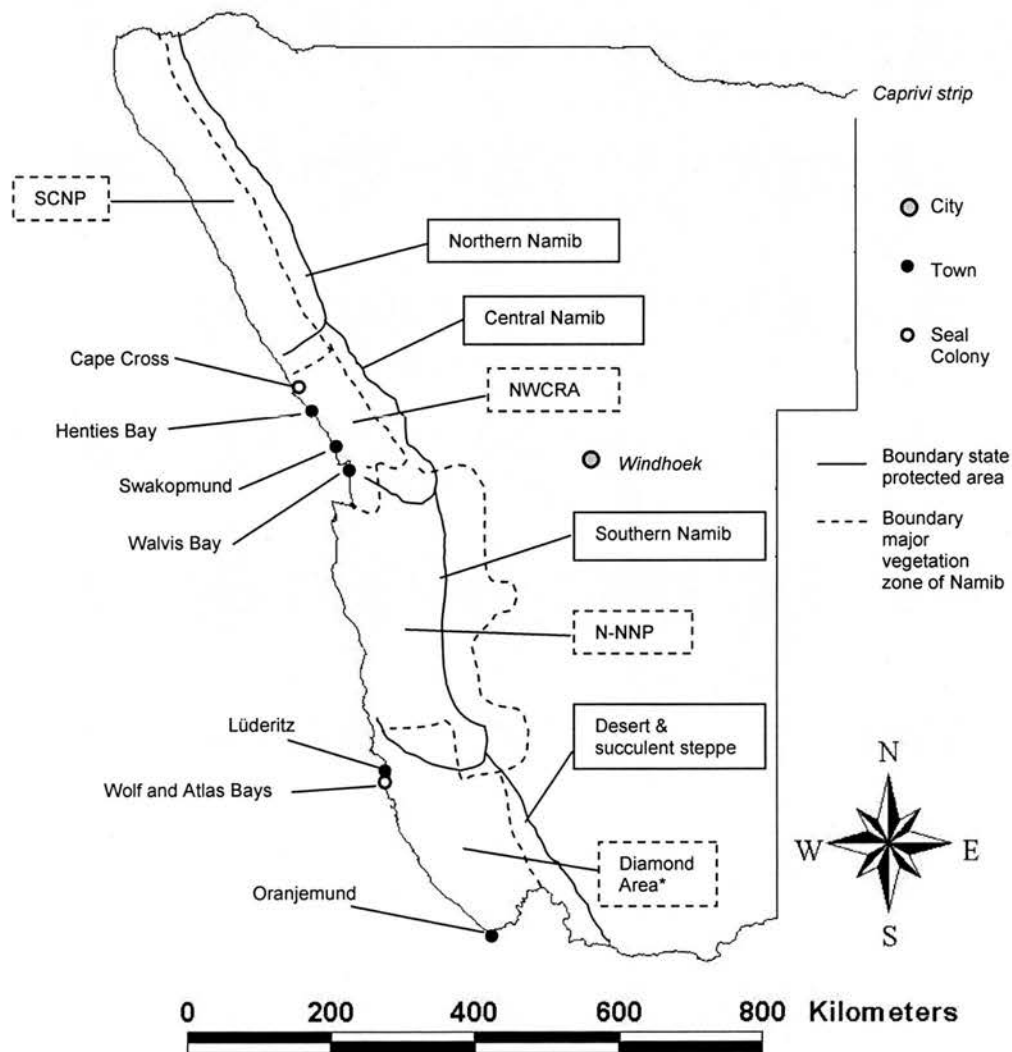
### **2.3.1 The Namib Desert coastline**

The coastal plains and the hyper-arid Namib Desert constitute the 2000km coastal geo-ecological zone which covers the entire length of the Namibian coastline and extends into Angola and South Africa. In Namibia, the eastern extent of this ecosystem, which covers about 15% of the country's land area, is not well-defined, reaching between 80 to 200km inland, roughly delineated by the 100-mm annual rainfall line or the Namib escarpment, at 1000m above sea level (Barnard 1998). The majority of the coastal area receives only between 0 and 50mm of rainfall (Barnard 1998) a year and the ephemeral rivers which reach the coast (the Omaruru, the Ugab, Koichab, Uinab and Hoanib) only flood in years of good rainfall (McIntyre 1998). Most of the moisture in this ecosystem originates from the coastal fog banks, which

form out at sea and move inland, supporting large lichen fields and hummock vegetation.

The Namibian coast can be divided into four broadly defined land types (Figure 2-1). The Northern Namib is an area of rugged mountains, gravel plains and dune fields which extends south, from the border with Angola, to the Ugab River (between 17°15'S and 21°08'S). The Central Namib consists largely of gravel plains, bound by the Kuiseb River in the south (23°07'S). The Southern Namib, which encompasses sand dunes, black rock outcrops and inselbergs reaches as far south as the northern limits of the Diamond Area, which consists largely of desert and succulent steppe (Barnard 1998).

**Figure 2-1: Map of Namibia illustrating the major land type zones and state protected areas.** 'SCNP' Skeleton Coast National Park, 'NWCRA' National West Coast Recreational Area, 'N-NNP' Namib-Naukluft National Park; \* The Diamond Area is currently not a National Park. Adapted from Barnard (1998).



The coastline is divided into four state protected areas (Figure 2-1), of which the Restricted Diamond Area Number 1 (Diamond Area) has yet to be designated as a National Park. The National West Coast Recreational Area, which encompasses Swakopmund and the Cape Cross Seal Reserve (CCSR), is the only area for which permits are not required for entry. This area is widely used by anglers and numerous camp sites are scattered along the coast (Jakkalsputz at Henties Bay, Myl 72, Myl 87, Myl 98 and Myl 108, the latter sites all named for the distance in miles from Swakopmund). The only major coastal routes are the short stretch of motorway linking Swakopmund and Walvis Bay and the C34, a salt road connecting Swakopmund and the Skeleton Coast Park. The Skeleton Coast Park, the CCSR and the Namib-Naukluft National Park allow permit holders access to a limited number of areas, beyond which only a few specialised tours are allowed to operate. Access to the Diamond Area is highly restricted as this is the site of Namibia's most productive diamond mines. Off-road driving is prohibited in all these state protected areas, vehicles being restricted to the few existing tracks.

Swakopmund and Walvis Bay, largely a tourist resort and an active industrial area and port respectively, are the largest of the coastal towns (Table 2-1); they are well connected by motorways to each other and the capital Windhoek.

**Table 2-1: Human population census data for the coastal towns.** Data from the 2001 Population and Housing census (NPC 2003).

Town	Population
Walvis Bay	43,611
Swakopmund	23,808
Lüderitz	13,295
Oranjemund	4,451
Henties Bay	3,285
<i>Total</i>	<i>88,450</i>

Henties Bay is a small retirement town approximately 60km south of Cape Cross which includes a seal processing factory and a few other businesses, its main activities being tourism and fishing, acting as a stop-over for tourists heading north or south along the coast. Lüderitz, once a prosperous diamond mining town is now

rapidly expanding as an industrial port and tourist attraction and is well connected to inland towns by motorway; traffic through this area is set to increase greatly once the Diamond Area is opened as a National Park. Oranjemund is a purpose-built town of the diamond mining company NamDeb (Pty) which controls access to this site and aside from tracks running through the Diamond area, there are no major routes connecting Oranjemund to other towns.

Off the Namibian desert coast lies an extremely productive and diverse marine ecosystem, supporting large and profitable fish stocks, central to the Namibian economy. The abundance of fish supports a large Cape fur seal population, last estimated at 1.5 million individuals (Wickens *et al.*, 1991). This marine system is driven by the cold Benguela ocean current, in which up-wellings bring nutrients from the deeper waters. This system also suffers from occasional anoxic conditions, thought to be responsible for a crash of the fish stocks in 1994 (Barnard 1998).

### **2.3.2 Study site selection**

Cape fur seal colonies are scattered along the length of the Namibian coast and jackals occur at all of these colonies (Stuart & Shaughnessy 1984, Wickens *et al.*, 1991). The seal colony of the CCSR (between 21°45'S and 21°53'S) and the colony stretching between the Wolf (26°49'S/15°07'E) and Atlas Bays (26°50'S/15°08'E) of the Diamond Area were selected as study sites for a number of reasons. Firstly, at over 160,000 individuals per colony (Pallett 2000, Mukapuli 2004), these are the largest Namibian colonies and they support correspondingly large and concentrated jackal populations, thus maximising the numbers of animals sampled and facilitating intra- and inter-specific observations. Secondly, these sites are the only locations where seals are harvested. The harvest permitted the sampling of a large number of individuals of both sexes and a range of age classes without the added logistical and financial constraints associated with trapping, anaesthetising and releasing seals. The seals are harvested yearly between July and mid-November with Government-set quotas for bulls and pups; the harvesting of adult females is prohibited. Thirdly, these sites were also selected for their proximity to urban domestic dog populations,

allowing for the investigation of behavioural interactions between the jackals and seals of the study colonies and domestic dogs.

Although seal colonies occur along the whole length of the Namibian coast, logistical and financial constraints precluded the inclusion of more isolated sites which were less likely to come into contact with domestic dogs, for comparison. Van Reenen's Bay (approx. 83km south of Lüderitz, 27°23'S/15°21'E) and Sandwich Harbour (approx. 53km south of Walvis Bay, 23°23'S/14°30'E) are further from towns but support much smaller colonies. The towns of Swakopmund and Henties Bay are approximately 60km and 128km from the Cape Cross colony respectively and Lüderitz is approximately 17km from the Wolf and Atlas Bay colony.

### **2.3.3 The Cape Cross Seal Reserve**

The CCSR is a small reserve (51km<sup>2</sup>) formed to protect the seal colony and the active guano platforms (Figure 2-2); there are no physical boundaries delineating the eastern extent of the reserve. The reserve itself consists primarily of sandy beaches, dune hummocks and large flat salt pans. The dune hummocks are covered with *Zygophyllum clavatum* and *Psilocaulon* succulents (Hiscocks & Perrin 1988), and separate the beaches and the salt pan. Beyond the main road (C34) the terrain turns into gravel hills and plains with very sparse vegetation.

Population estimates for each colony had to be estimated from total pup counts as no other data were available at the time of writing; pup counts were multiplied by a factor of 5 (Pallett 2000). The Ministry of Fisheries conducts yearly aerial surveys of most of the colonies and in 2001 estimated the pup population of CCSR at 37,394 (Mukapuli 2004). Hence the colony was estimated at approximately 187,000 pups, cows and bulls in 2001. This colony may stretch up to 10km south along the beach from the rocky promontory when the colony expands to include both bulls and pups in the summer months<sup>1</sup>. The bulls only haul-out in late October to early January

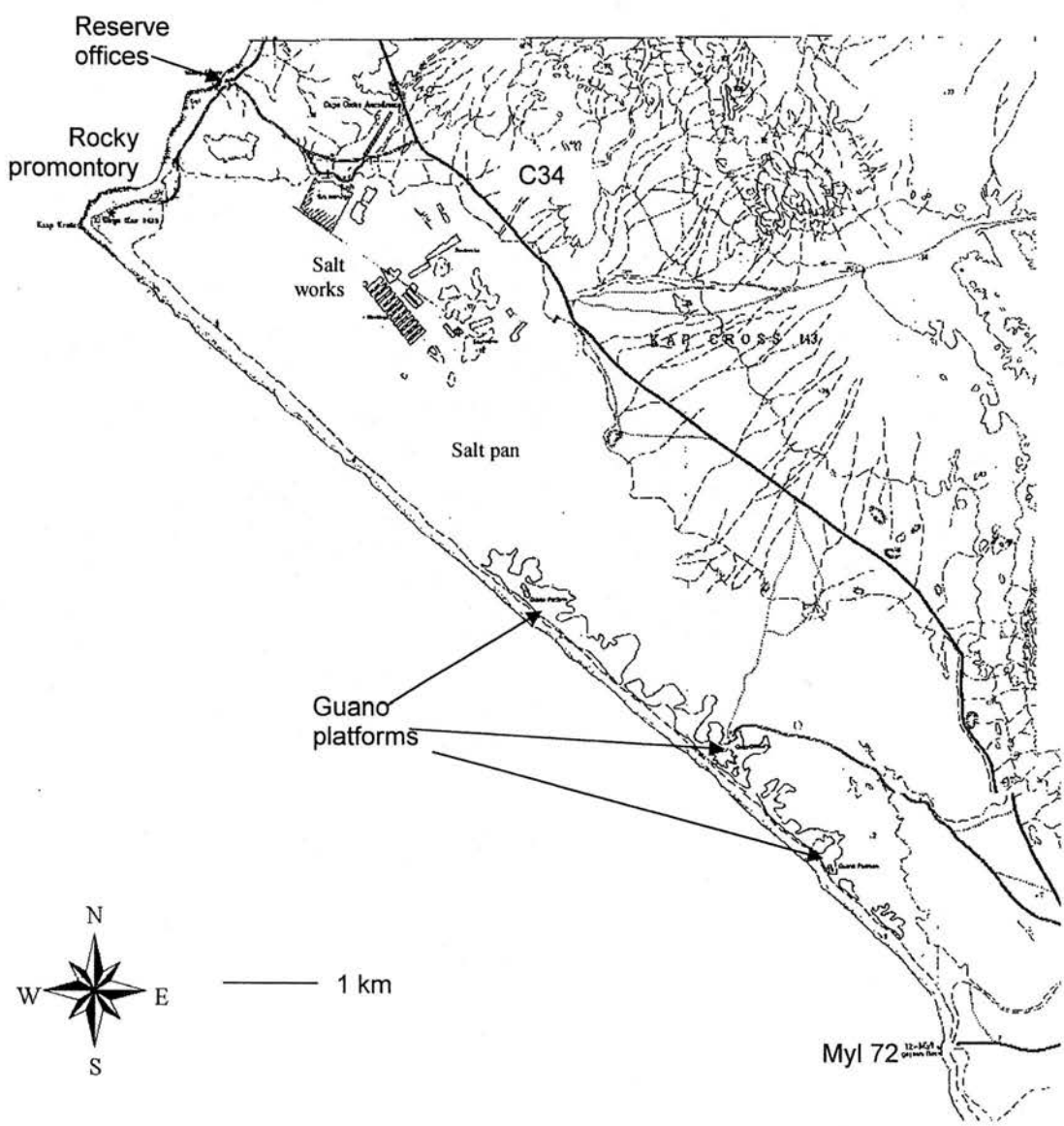
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<sup>1</sup> Summer months: November to April, Winter: May to October



which is when the colony is at its largest (Apps 2000, Pallett 2000, Macdonald 2001, Berry 2002).

**Figure 2-2: Topographic map of the Cape Cross region.** Between 21°45'S and 21°53'S. Surveyor General, Windhoek (1972), 1:50,000 2113DD, Kaap Cruis.



Apart from the black-backed jackal, the brown hyena is the only other large mammal species in the reserve, comprising a very small population thought to be between 8 to 10 individuals (pers. comm. I. Wiesel, research scientist, Brown Hyena Research Project). The reserve also includes a series of small lagoons with three active guano platforms which host large numbers of a wide variety of bird species including lesser

flamingos (*Phenicopterus minor*), cape cormorants (*Phalacrocorax capensis*), white breasted cormorants (*P. carbo*), and gulls (*Larus* spp.). An active salt mine occupies an area of approximately 6km<sup>2</sup> in the north-east corner of the reserve.

The reserve is run by the Ministry of Environment and Tourism (MET) with a permanent Ranger and two assistants living on site and is open to tourists 7 days a week, all year round. There is also a small privately-owned hotel with approximately 20 staff, on the northern border of the reserve near the offices.

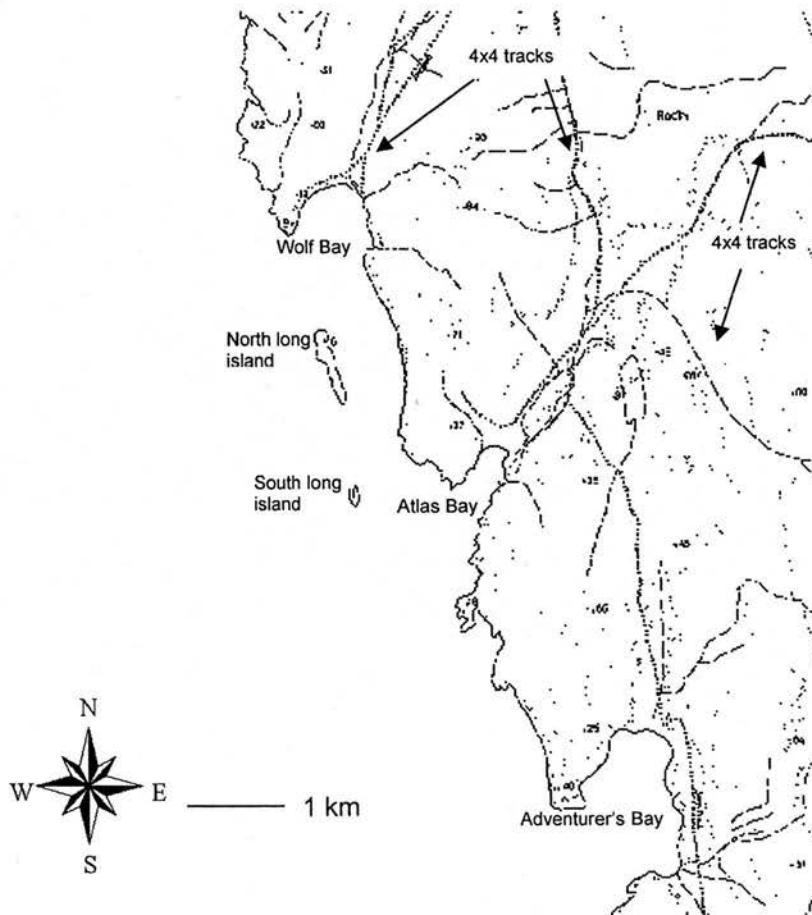
### **2.3.4 The Diamond Area**

The Diamond Area is a vast area of approximately 26,000km<sup>2</sup>, bordered by 250km of coastline on the west and extending up to 100km inland. The landscape is very varied, a combination of desert and succulent steppe, including vast gravel plains, dune fields and rocky mountains. The Diamond Area has the highest biodiversity in Namibia, supporting over 700 plant species, and is one of the major refuges for IUCN (The World Conservation Union) Red list mammals including the aardwolf (*Proteles cristatus*) and the bat-eared fox (*Octocyon megalotis*), which occur inland from the coast in the central region of the Diamond Area. But this area has also been invaded by a number of alien species introduced by humans which may also pose a disease risk to the native fauna. These comprise the house mouse (*Mus musculus*), the black rat (*Rattus rattus*), the European rabbit (*Oryctolagus cuniculus*), the domestic cat (*Felis catus*), the African wild ass (*Equus asinus*), the feral horse (*Equus caballus*) and domestic dogs (Barnard 1998).

The Wolf and Atlas Bays (Figure 2-3) themselves are small sandy beaches surrounded by rocky outcrops and gullies, which run inland into a landscape of rocky hills, saltpans and desert plains. The seal colony at this site can be estimated at approximately 167,000 individuals, using the pup population estimate of 2001 ( $n=33,377$ ) (Pallett 2000, Mukapuli 2004). But as at Cape Cross, the only other wild mammal species in the vicinity of the coast and the seal colonies are the jackals and

brown hyenas, the brown hyena population estimated to be between 11 and 17 individuals in 2000-2001 (I. Wiesel, research scientist, unpublished data).

**Figure 2-3: Topographic map of the Wolf and Atlas Bays of the Diamond Area.** Between 26°47' S and 26°52' S. Surveyor General, Windhoek (1972), 1:50,000, 2615CC Elizabeth Bay.



## 2.4 Field periods and permits

**March  
2001**

During the first field period the seal colonies of Möwe Bay (c.250km north of Cape Cross in the Skeleton Coast Park, 19°23'S/12°43'E), Cape Cross, Wolf and Atlas Bay and Van Reenen's Bay were visited and their suitability as study sites assessed. The 4x4 vehicle tracks, resting sites, hyena latrines, dens and heavily used trails in the vicinity of the seal colonies were mapped for Cape Cross, the Wolf and Atlas Bays and Van Reenen's Bay in order to assess the carnivore activity in the area and to scout accessible capture areas. A number of non-invasive techniques for the determination of the jackal population size were also piloted, namely the scent station technique and the collection of hair and faecal material for genetic analyses. Interviews were conducted with research scientists, Ministry of Environment and Ministry of Fisheries staff working in the West Coast Recreational Area, the CCSR, and the Diamond Area and with veterinarians working in Swakopmund and Lüderitz, to obtain information on jackal and seal population sizes in the areas and to set-up informal networks for the collection of dog blood samples.

**May**

**September**

*Sampling of jackals, domestic dogs and seals.* Pilot jackal capture sessions were carried out at both study sites and radio-tracking performed at Cape Cross. Domestic dogs from Lüderitz and Walvis Bay were also sampled and seal sera obtained from the harvests at Wolf and Atlas Bay and Cape Cross. Pilot behavioural observations were made at Cape Cross and Van Reenen's Bay.

**December**

**May  
2002**

*Sampling of jackals, domestic dogs and seals.* The majority of the data collection occurred during this period but field activities and sampling at both study sites were affected by a CDV epidemic. For logistical and financial reasons, the jackal sampling and data

**March  
2003**

collection (capture, radio-tracking, behavioural observations and post-mortems) were concentrated at Cape Cross with only jackal capture occurring in the Diamond Area.

**October  
2003**

A brief *jackal capture* period was undertaken by S. Funk and N. Jenner (IOZ Jackal Project) at Cape Cross.

A summary of the sampling with details of the species sampled and the samples collected from each study site is given in Table 2-14 on page 81.

### *Permits*

Ethical clearance for the use of the capture equipment was obtained from the Institute of Zoology Ethics Committee. Permission to capture and sample black-backed jackals throughout Namibia and to sample Cape fur seals at the Cape Cross and Diamond Area colonies was granted by Dr. H. Kolberg, Chief Conservation Scientist at the Ministry of Environment and Tourism (Windhoek, Research and Collection Permit Nos: 549/2001/2002-3 ). Permission to sample the Cape fur seals during the harvests at Cape Cross and in the Diamond Area was granted by the Ministry of Fisheries and Marine Resources by the Director of Resource Management Dr. Oelofsen (Windhoek) and the regional Director, Dr. B. van Zyl (Swakopmund). Temporary veterinary permits for the capture and anaesthesia of black-backed jackals were granted to project veterinarians, Drs. S. Di Concetto and G. Vila-Garcia, by Dr. C. Bamhare, Principal State Veterinarian of the Veterinary Council of Namibia. Permission to use the 173 MHz frequency on the Telonics TR-4 unit for radio-tracking, was granted by the Namibian Communications Commission (Windhoek), under the Radio Act (Act. 3 of 1952).

Licence for the export of jackal and dog serum and tissue samples was granted by the Ministry of Environment and Tourism (Windhoek). A CITES import permit for the Cape Fur Seal serum samples was granted by the Department for Environment, Food and Rural Affairs Wildlife Licensing and Registration Service (Permit No. 260487/01) and the permit for export of CITES samples from Namibia was granted

by the Ministry of Environment and Tourism's Scientific Services Directorate (Windhoek, Permit No. 47539). The import licence for Institute of Zoology, London was granted under licence number AHZ/2051/2001/4, the Animal Health Act 1981, the Importation of Animal Pathogens Order (1980).

## ***2.5 Sampling of jackals***

### **2.5.1 Jackal Capture**

#### ***2.5.1.1 Cage traps***

Four cage traps were constructed (60x70x150cm) with round metal bars (diameter 1.2cm), and closed by 6cm diamond mesh fencing. The cages were designed to be small enough to discourage brown hyenas from entering but large enough for jackals. The cage was accessed by a sliding door at each end, only one of which functioned as a trap door which was released when the animal stepped on a treadle plate positioned at the opposite end of the cage. The door was held open by the pressure of its own weight resting on a bar connected to the treadle plate. The bait was placed beyond the footplate which was wide enough that the animal had to step on it to access the bait. The floor of the cage was covered with a thick layer of sand and the area around the trap made to look as natural as possible.

#### ***2.5.1.2 Foothold traps***

Padded steel foothold traps (No.4 Victor Softcatch, coil spring, Woodstream Corporation, Lititz, Pennsylvania, USA) were used in order to increase capture success. Each trap was equipped with a 12cm chain, shock spring and swivel to reduce the chances of injury to the animal by providing some elasticity to the connection between trap and its site of anchorage. Each trap was secured by a swivel and screw lock to a 75cm, 8mm straight link chain, and anchored using 2 double stake swivels with 4 cross-staked rebar stakes (1.2x60cm). This set-up provided



sufficient anchorage to hold the largest animal in the area (i.e. brown hyenas), if they were to become trapped. The trap was set in a shallow depression and a square of tissue paper placed over the foot plate in order to prevent sand blocking the mechanism. The trap was then covered with a fine layer of sand. Traps were only placed where they could be approached from only one direction, for example, at the base of a dune hummock and stones, twigs or other objects were usually used to guide the animal's steps. Traps were baited with the meat placed behind the trap, roughly at the distance between the jackals' head and front paw. Some un-baited traps were also placed along the 'jackal highways' – clearly visible and frequently used routes between the colony and inland resting sites (Hiscocks & Perrin 1988). Care was taken not to place foothold traps near active dens.

#### *2.5.1.3 Bait*

The most frequently used bait was large pieces of seal carcass, kindly provided by the seal concessionaires of Lüderitz and Cape Cross. If seal could not be obtained fresh chicken pieces were used. A trail of blood from the bait was usually left between the plate and the area around the cage entrance.

#### *2.5.1.4 Cage trap 'habituation'*

Cage 'habituation' describes the setting (the cages were fixed open so that they could not be triggered) and baiting of the cages for the assessment of the level of 'trap-wariness' of the jackal population at the two study sites. Because the jackal population of the coast is not persecuted, it was assumed that capture success would be high, compared to sites inland where jackals are often shot or poisoned. The habituation was not thought to deter jackals as to negatively affect the capture success – the provision of freely-accessible bait likely increased capture success.

Cages were 'habituated' before capture in 2001 for a number of reasons. Firstly to ascertain what level of capture success this type of method was likely to achieve. If the jackals were too wary of entering the cage, another method would have to be

selected; secondly, to assess the chances of capturing brown hyenas, the only non-target species likely to enter the cages; lastly, the cages were also 'habituated' to assess the suitability of different capture sites.

Cages were fixed open and bait was placed inside. The area surrounding the cage was swept free of debris and prints and the trap left open for approximately 12 hours overnight. The following morning, cages were subjectively scored on a scale from 0 to 5, where '5' was a very well visited cage from which all bait had been removed, and which showed numerous signs of activity including prints and digging. A cage which had not been approached, around which no prints could be found, scored '0'. In the case of a low score, either a second habituation attempt was made in which a bait trail, consisting of strong-smelling chicken or fish broth, was left leading up to the cage, or the cage was moved to another site.

#### *2.5.1.5 Trap placement, setting and monitoring*

The cage traps were positioned at an average distance of 1.5km apart to increase capture success for each cage and to reduce the chances of other jackals encountering a trapped individual. Foothold traps were set in small clusters of up to 4 traps (no more than 4 clusters were set at any one time) over an area small enough to enable visual monitoring of each cluster. The traps were placed at least 100m from the seal colony in order to avoid disturbing the seals and close enough to the car tracks for moving the cage traps. In order to coincide with peak activity periods, traps were usually set between 4am and 5am and shut by 11am for morning sessions and opened from 6pm to 2am for over-night trapping sessions.

Once set, cage traps were checked visually every 45 to 60 minutes and foothold traps continuously or every 30 to 45 minutes, using binoculars and spotlights. In 2001, a radio alert system was tested on the cage traps. Although adaptable for cage and foothold traps, this system was not used in 2002 due to the damage sustained by jackals gnawing at the system's components and the frequent interruption of the signal from poor reception.

### 2.5.1.6 Jackal anaesthesia

In 2001 and for the first capture period in 2002, the veterinarian from Swakopmund (Dr. H. Winterbach, veterinarian, Swakopmund) assisted with capture and provided training for project staff, after which two project veterinarians (Drs. G. Vila Garcia and S. Di Concetto), assisting with research on sarcoptic mange and undertaking a project on anaesthesia respectively, were responsible for all anaesthesia and euthanasia.

In 2001 and the first capture period of 2002, jackals were anaesthetised (IM) with Tiletamine-zolazepam (Zoletil 100, 100mg/ml, Palmvet Services, Rynfield, South Africa) at a dosage of 10mg/kg. At Cape Cross, in the subsequent capture periods of 2002 and 2003 jackals were anaesthetised (IM) with one of three different drug combinations (listed below) as part of a study to identify the most suitable reversible protocol (Di Concetto *et al.*, in prep.):

- a) Ketamine (Anaket-V 100mg/ml, Bayer (Pty) Ltd., South Africa) 3-5mg/kg and Medetomidine (Domitor 1mg/ml, Novartis South Africa (Pty) Ltd.) 0.04-0.05mg/kg,
- b) Ketamine 3-4mg/kg, Diazepam (Tranject 10mg/2ml, SCP Pharmaceuticals (Pty) Ltd., South Africa) 0.2-0.5 mg/kg and Medetomidine 0.03-0.05mg/kg or,
- c) Tiletamine-Zolazepam (Zoletil 100, 100mg/ml, Palmvet Services, Rynfield, South Africa) 2-4 mg/kg and Medetomidine 0.03-0.05 mg/kg.

As part of the anaesthesia study, detailed information was recorded for the assessment of the depth of anaesthesia and the animal's condition as assessed by oxygen saturation of the blood, the ease of intubation, heart and respiration rates, body temperature, eye position, and palpebral and pedal reflexes, jaw tone and other observations (Appendices, Chapter 2, section 8.1.2, Anaesthetic sheet). Oxygen saturation was measured using a pulse oximeter (Nellcor N-20, Nellcor Inc., Hayward, USA). In 2003, the Anaesthetic sheet was expanded to include additional details of the anaesthesia, namely systolic and diastolic pressure, mean arterial

pressure, protrusion of the 3<sup>rd</sup> eyelid, pupil dilation, and anal reflex and 3-4 electrocardiogram readings.

In the Diamond Area in 2002, jackals were anaesthetised with Medetomidine and Ketamine in dosages given in 'a' above. If additional time was required to complete the sampling protocol, top-ups were administered – 0.2ml of Tiletamine-Zolazepam (IM) for those animals anaesthetised with Tiletamine-Zolazepam alone and 0.1ml Ketamine (IV) for those which had received Ketamine in the initial anaesthesia. Reversal of the anaesthesia with Medetomidine was performed with Atipamezole (Antisedan 5mg/ml, Novartis South Africa (Pty) Ltd.) at a dosage five times higher than Medetomidine. Wherever possible, jackals were placed in cages to recover from the anaesthetic and only released when no longer ataxic and fully alert. If a cage was not available jackals were tightly wrapped in a blanket and placed in a sheltered site, such as a dug out resting site, to recover and checked on at regular intervals.

### **2.5.2 Jackal sample and data collection**

Sedated jackals were covered with a blanket during sampling, muzzled and blindfolded; if the eyes were open, Lacri-Lube eye ointment was applied to prevent desiccation of the cornea.

In 2001 each jackal was sampled for blood (for serum), weighed, measured, the sex determined, and an ear snip (preserved in 100% ethanol) taken for genetic analyses and the following body measurements taken (in cm): nose to shoulder, nose to anus, tip of tail bone to base of tail, skull length, shoulder height, hind hock to tip of pad and length of hind pad. Up to 8ml of blood was sampled from the cephalic vein. Thorough searches were made for injuries (which were sprayed with oxytetracycline (Terramycin aerosol spray, Animal health Pfizer Ltd., UK)) and ectoparasites, areas with mange lesions were noted and a record made of reproductive status. Any parasites recovered were stored in 10% buffered formalin and donated to the Curator of Mammals of the National Museum of Namibia, Mr. S. Eiseb.

All blood for serum was refrigerated, left to clot and centrifuged for serum within 12 hours. In the field, sera were frozen at -10°C and later transferred within 3 months of sampling, by portable freezer, to the CVL (Central veterinary laboratory, Windhoek) for storage at -20°C. Samples were shipped to the UK on ice and stored at -20°C until used. All samples were heat inactivated at 56°C for 30 minutes prior to testing; if samples were frozen between tests, they were inactivated again at 56°C for 10 minutes as required by the Intervet (UK) laboratories. Between sampling and the first testing samples did not undergo more than 2 freeze-thaw cycles.

In 2002 the sampling protocol and data sheets (Appendicies, Chapter 2, section 8.1.1, Jackal capture data sheet) were extended to include notes on recovery, condition, and additional samples and data as follows:

1. *Blood in anticoagulant*: 0.5ml of blood in anticoagulant (EDTA, B-D sterile vacutainer, UK) for blood slides. This was kept refrigerated until use.
2. *Skin biopsy of a mange lesion*: A skin biopsy from a mange lesion taken with a sterile disposable 8mm biopsy punch and stored in 10% buffered formalin. The wound was sealed with a surgical adhesive (Vetbond, 3M Animal care products, USA) and sprayed with oxytetracycline.
3. *Skin scrape of a mange lesion*: A skin scrape from a mange lesion area taken with a No. 10 scalpel blade and stored in 10% buffered formalin. Scrapes were made deep enough to draw droplets of blood and sprayed with disinfectant.
4. *Diagrams of mange lesion areas*: Dorsal and ventral schematic drawings of mange lesion areas, with and without alopecia, with a record of the predominant stage of the mange infection on a scale from 0-5 (0 none, 1 crusty, 2 not crusty, 4 recovering and 5 unknown).
5. *Faecal samples*: Fresh faecal samples, approx. 2g in 100% ethanol (7ml) and 2-4g in 10% buffered formalin (25-35ml) as measured with a wooden spatula, for genetic and parasitological analyses respectively.
6. *Nasal swab*: A nasal swab, taken with a sterile swab and refrigerated. All swabs were submitted to Dr. G. Eberle at the CVL (Windhoek) for culture and identification.

7. *Fur clipping*: A small clipping of hair for stable isotope analysis of the marine and terrestrial components of the jackals' diet (J. Roth *et al.* in prep, University of California at Florida).
8. *Data on condition*: A record of the fat reserves (thin, lean, optimum, obese, gross) and condition (over or underweight). Each individual was given an overall score on a scale of 1 to 7: '1' for thin to emaciated and underweight, '2' for thin to lean and underweight, '3' for lean and underweight, '4' for lean to optimum, '5' for optimum, '6' for obese and overweight and '7' for grossly overweight.
9. *Clinical Signs*: Records were also made of any clinical signs consistent with CDV infection. Each individual was examined for the occurrence of any one of the following signs: ataxia, myoclonus, seizures, ocular-nasal discharge, respiratory signs using auscultation. Anorexia, coughing and diarrhoea (although they did occur in many cases) were not included as it was not possible to assess individuals for these conditions during capture or anaesthesia. Any CNS signs occurring during sedation and recovery from the anaesthesia were not recorded as these may have been caused by the anaesthetic drugs. A suspected case of canine distemper was defined as an individual positive for one or more of the above clinical signs.

Due to the limitations associated with diagnosing CDV infection solely on one or more clinical signs this information was used in conjunction with other data to confirm the occurrence of a CDV epidemic, lab tests were used to detect exposure and infection in cases with clinical signs, and the interpretations based on the observations of clinical signs alone treated with caution.

In 2003, only sera and age class, sex and body weight data were available from each individual, together with information on the presence or absence of clinical symptoms consistent with CDV infection.

The blood smear technique (Cowel 1999) was used to make slides for a rapid Giemsa stain using the Rapi-diff II stain pack (Triangle biomedical sciences Ltd, UK). Slides were air dried and stained by immersion for 15-30 seconds in each of three solutions.



All slides were submitted for examination (for viral inclusions and blood parasites) and for differential white blood cell counts, to Ms. M. Kotze at the CVL (Windhoek).

#### *2.5.2.1 Identification of individual jackals*

In 2001, jackals were fitted with either a radio collar (Biotrack Ltd., Dorset, UK) or a visual collar. Visual collars were hand made from leather machine belting (3cmx3mm) and colour-coded with heat-shrink tubing (Farnell Electrical Components Ltd., SP254, 2:1 heat-shrink ratio, diameter when shrunk 12.7cm) in 2 or 3 colour combinations which were clearly visible from both sides of the animal; radio collars were also given identifying colours. Due to the loss of the collars and the heat shrink, in 2002 the collars were replaced with one or two coloured numbered ear tags (Dalton tags UK, Jumbo tags), one per ear. Jackals were identified by a label comprising the animal's sex (F, female or M, male), age class (J, S or A, see Ageing of jackals below), the number on the ear-tag of right ear, the ear-tag colour and the location (C for Cape Cross or L for the Diamond Area). Only jackals at Cape Cross were fitted with radio-collars as the small sample size and terrain of the Diamond Area study site rendered tracking impractical.

#### *2.5.2.2 Ageing of jackals*

The analysis of cementum layers for the ageing of jackals was not used as the extraction of teeth from captured individuals was not desirable and although a canine was sampled from every carcass, analysis of the cementum layers was not possible due to financial reasons. Furthermore, it is thought that the analysis of cementum layers is of no value in determining age after one year (Bingham & Purchase 2003). Tooth wear, as described by (Lombaard 1971) has been used to age jackals in other studies (Kaunda 2001, Loveridge & Macdonald 2001) and Ferguson *et al.*, (Ferguson *et al.*, 1983) combined tooth wear and body weight to define three age classes as follows:

- *Juveniles (<12 months)*: animals less than one year of age (those captured before the 1st of August). Jackals at this age are fully grown but slender, usually weighing less than 6kg.
- *Sub-adults (12-35 months)*: animals over one year of age but less than 3 years. Jackals are usually fully grown at just over one year (Apps 2000).
- *Adults ( $\geq 36$  months)*: animals 3 years or older, usually weighing more than 6kg and stockier in build than sub-adults or juveniles.

Jackals in this study were assigned to one of the three age classes described above on the basis of the tooth wear as described by Lombaard (1971). The wear on the upper incisors of an individual was compared to photographs of wear in individuals from 1 to 7 years of age, provided in Lombaard (1971). If tooth wear could not distinguish between a juvenile and a sub-adult, body size (i.e. slender or stocky in build) and weight were also taken into account. Jackals between 1 and 3 years of age were not classified as adults because although they are sexually mature at 11 months, the social system delays reproduction until the individual has found a mate and established a territory (Moehlman 1983, Apps 2000).

#### 2.5.2.3 *Jackal post mortems and tissue sampling*

For each carcass, the same details were recorded as for a capture (Appendices, Chapter 2, section 8.1.3, Jackal *post mortem* data sheet) but with added information on the condition of the carcass (Table 2-2), the time of death and the cause of death, if known. In the *post mortem* a veterinarian examined the oral cavity and internal organs for abnormal colouration, swelling and other gross pathological changes. The project veterinarians also provided training for the project staff in order that they may conduct post-mortem examinations in their absence, if necessary.

**Table 2-2: The categories for the different stages of decomposition of jackal carcasses.** As listed on the post-mortem sheet (Appendices, section 8.1.3).

Category	Description
Extremely fresh	As if just died, no bloating
Slight decomposition	Slight bloating, blood imbibition visible
Moderate decomposition	Moderate bloating, skin peeling, organs beginning to deteriorate but still recognisable
Advanced decomposition	Major bloating, skin peeling, organs beyond recognition, bones exposed due to decomposition
Intermediate	No organs present, skeletal remains, may be mummified

Depending on the condition of the tissues, the following samples were preserved in 10x the tissue volume (less than 1cm<sup>3</sup> for virus isolation) of 100% ethanol and 10% buffered formalin (for histopathology):

- bladder, spleen, testes or ovaries, lymph nodes (mesenteric and/or cervical), liver and lung.

Where possible and if the individual organs were in good condition, the following additional tissues were sampled:

- bronchial lymph nodes, adrenal glands, brain (sections of cerebrum and medulla or half of the brain sectioned along the sagittal crest), heart, kidney, and intestine.

It was not possible to obtain a full complement of organ tissues from each animal due to the degree of putrefaction, the limited amount of equipment and supplies available, and the limitations in technical knowledge early on in the study.

In addition to carcasses collected from the field, carcasses were obtained from road kills and following the euthanasia of free-ranging moribund or captured jackals. Jackals were euthanased either by injection (IV) of Pentobarbitone (150mg/kg) by a veterinarian or by shooting. Jackals were shot by Ministry of Environment officials. The euthanasia of animals *in extremis* was performed in accordance with instructions from the Ministry of Environment which requires the euthanasia of animals

considered to be suffering from severe disease (e.g. sarcoptic mange or canine distemper), broken limb(s) or other severe injuries.

Carcasses and moribund animals located in the study areas were reported by a number of informants (see collection of data by personal communication, section 2.8). Whenever possible the carcasses were recovered for sampling but if this was not possible, as much data as possible was recorded on the location and condition of the carcass. The daily activities of the project, including radio-tracking, behavioural observations and capture also provided reasonable coverage of the study areas for the location of carcasses. The shorter periods spent in the Diamond Area and the rocky terrain did not allow for as systematic and a thorough search as at Cape Cross. Carcasses were not collected during, and there was no data available on any carcasses from, the field trip in October 2003.

### **2.5.3 Jackal population size**

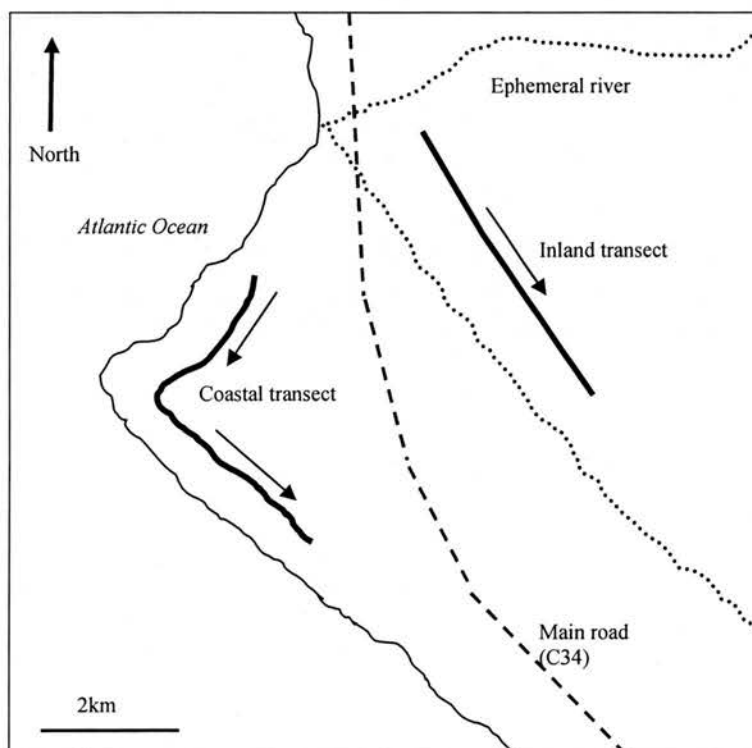
A number of methods were assessed during the first field period. The scent station technique has been used for a number of species (Linhart & Knowlton 1975, Roughton & Sweeny 1982, Conner *et al.*, 1983, Diefenbach *et al.*, 1994, Berg 1999) and was piloted for jackals at Cape Cross. This method was not considered suitable due to the time-consuming setup, the low proportion of stations that were visited and the quality of the terrain (large areas of hardened earth or sand crusted with salt). The collection of faecal material or hair for the extraction and sequencing of genetic material has been performed for a number of species (Anderson & Haroldson 1996, Gagneux *et al.*, 1997, Goossens *et al.*, 1998, Taylor *et al.*, 1998, Kohn *et al.*, 1999, Sloane *et al.*, 20001) and is an effective non-invasive method for the determination of population size. Unfortunately, the processing of faecal samples for genetic analysis was not possible due to financial constraints and the collection of sufficient hair samples was judged unfeasible for this species.



### 2.5.3.1 Jackals: distance sampling

The limited time spent in the Diamond Area and the practical difficulties associated with sighting jackals in the rocky terrain of the study site made estimations based on sightings impractical as the sample size would have been too small. Hence the distance sampling method (Buckland *et al.*, 1993, Sutherland 1996) was piloted at Cape Cross during the third field period between October and September 2002. Two 5km transects (Figure 2-4) were set up with marker posts at 500m intervals, one along the length of the seal colony and one in parallel, 5km inland.

**Figure 2-4: Map of the Cape Cross area showing the position of the inland and coastal distance sampling transects and the direction of sampling.**



Transects were walked at a constant 5km per hour; no off-road driving is allowed in the reserve and there were no suitable tracks. Transects and starting times were selected at random. Transects either started during the jackals' activity period (between 09:30 and 10:00) or during the non-activity period (at 16:00). The two transect positions and the different starting times were selected in order to compare

counts at different times of day, as it had been proposed that most jackals move to the hills further inland between activity periods (Hiscocks & Perrin 1988). The observer used a GPS to follow the transect line and to record his or her position when a jackal was sighted. The distance in meters to the sighted jackal was estimated and the angle to the jackal's position measured using a compass.

The distance sampling method was not selected as the basic assumptions of this method were violated when the animals moved before they were sighted and the behaviour of one jackal, running from the observer, affected the behaviour of others in the area. In addition, the high density of jackals during the activity periods made it very difficult to record the necessary data for each individual. But some of the data collected from the distance sampling transects was used to design the strip transects.

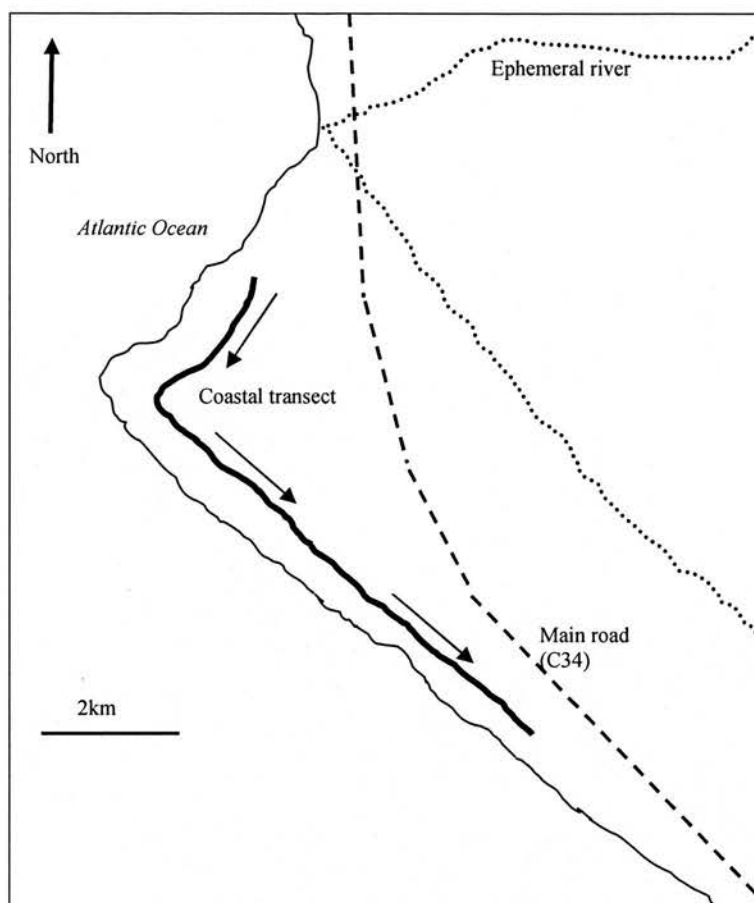
#### *2.5.3.2 Jackals: mark re-sight, strip transects*

The mark and re-sight methodology was selected in light of the successful capture and release of 32 tagged jackals at Cape Cross in 2002. From the pilot distance transects, it was possible to determine that the maximum distance from the transect line at which a jackal could be identified (using binoculars) as tagged or radio-collared was 250m. On the basis of the low number of sightings on the inland transect and prior radio-tracking of jackals in the area (Hiscocks & Perrin 1988) it was concluded that the majority of the population was present in the colony area during the activity periods.

The existing coastal transect line, positioned 250m from the edge of the seal colony was extended with a second strip transect (Figure 2-5) in order to cover the entire length of the seal colony (the jackals' foraging area). Starting times were between 06:10 and 07:05 for activity periods, depending on the visibility, and between 14:00 and 15:00 for non-activity periods.



**Figure 2-5: Map of the Cape Cross area showing the position of the extended coastal transect.**



The location (closest transect marker as determined by the GPS) and time of each sighting was recorded. In order to obtain total counts for both the visible area along the transects and within the strip transects, jackals beyond 250m were recorded as unidentified and those within the 250m boundary either side of the transect were recorded as tagged or not tagged. The total numbers of jackals observed within each transect were used to calculate the average jackal density at the colony in the activity and non-activity periods.

In order to keep the time taken to complete the transects to a minimum and to reduce the bias associated with jackals being re-counted further along the transect line, transects were limited to 5km. The transect section (first or second 5km section) and the starting time (activity or non-activity period) were selected at random. The observer walked at a constant speed of 5km per hour.

Population estimates were obtained from the numbers of marked and unmarked individuals sighted during activity periods using the joint hypergeometric maximum likelihood estimator (JHE) in NOREMARK (White 1996a). There are a number of assumptions to be met a) The number of marked animals is assumed to be the same for each survey although the probability of sighting animals is not b) The probability of sighting each animal in a survey is the same c) All marked animals are correctly identified, counted and recorded and d) Animals must not lose their markings (ear-tags). Non-activity period transects were not used as the lower number of sightings indicated that some of the population may have left the transect area, thus violating the assumption of the JHE that all marked animals are in the survey area for each survey (White 1996b).

Since jackals are known to occur at all breeding and non-breeding seal colonies in Namibia (Stuart & Shaughnessy 1984), the ratio of seal pups to jackals at Cape Cross was applied to the seal pup population estimates of the Diamond Area study site (Mukapuli 2004) and the coast as a whole. This seal pup:jackal ratio was calculated from the point estimate of the seal pup population at Cape Cross (Mukapuli 2004) and the JHE estimate of the jackal population. The 95% CI values of the Cape Cross jackal population estimate were also used to calculate the 95% CI for the jackal population estimates.

## **2.6 Sampling of domestic dogs**

### **2.6.1 Sources of domestic dogs**

The project was largely dependent on the Town Councils' round-up of feral and unclaimed neighbourhood dogs for euthanasia for opportunities to sample dogs for serum and for access to carcasses; the Town Council advertised prior to round-ups so that owned dogs were kept off the streets for the day of the collection. Collaborations were set up with the Town Councils of Swakopmund and Lüderitz, the Lüderitz SPCA (Society for the Prevention of Cruelty to Animals), the private and state veterinarians visiting Lüderitz, and the veterinarian responsible for the euthanasia of feral dogs in Swakopmund and Walvis Bay (Dr. H. Winterbach).

Veterinarians were asked to collect serum and basic information on condition, age and sex from unvaccinated dogs visiting the clinics, or those seen outside of the clinic. If any of these dogs was euthanased due to suspected CDV infection, veterinarians were asked to collect tissues (bladder, spleen, testes or ovaries, mesenteric lymph node, liver, brain and lung) in 100% ethanol and 10% buffered formalin.

In order to increase the sample size from Swakopmund, a sampling of 'neighbourhood' dogs (Perry 1993) was organised at the local rubbish dump and the Democratic Resettlement Community (DRC), a settlement on the edge of town, in November 2002. This was in collaboration with the local Town Council Dog Officer, responsible for the enforcement of registration procedures and the round-up of stray animals. Animals caught by their owners were sampled for serum and basic data as described above.

The occurrence of a number of confirmed rabies cases during the CDV outbreak in Lüderitz in January 2003 (Chapter 3), prompted the local SPCA and Town Council to organise a door-to-door and point rabies vaccination campaign for the administration of free vaccine. Dog owners contacted the SPCA officer to book a

vaccination after which a visit was made to the house or owners attended vaccination points set up in town after advertisement in the local paper and radio. If permission was granted by the owner, dogs were sampled for blood and basic data collected, including information on vaccination history and health at the time of sampling.

Hence dog serum samples were collected from several different sources on a largely opportunistic basis:

1. Town council round-up of stray dogs for euthanasia
2. Veterinarian sampling of unvaccinated dogs at clinics
3. A visit to the Swakopmund town rubbish dump and DRC
4. Sampling during a rabies vaccination campaign in Lüderitz.

The tissues for the histopathological diagnosis of CDV infection were sampled from carcasses obtained via the Town council round-ups, suspected cases of CD taken to the veterinarians' clinics and carcasses encountered at owners' homes in Lüderitz during the vaccination campaign.

### **2.6.2 Domestic dog sample and data collection**

The samples collected from domestic dogs were processed as for those collected from jackals (see section 2.5.2). Live dogs sampled in 2001 were sampled for between 5-10ml blood for serum from the cephalic vein, and the following data recorded for each individual: age class, location and the presence or absence of mange lesions; dogs were muzzled and manually restrained during blood-taking. In 2002 and 2003 sample and data collection were expanded to include the following data and samples:

1. *Blood in anticoagulant*: 0.5ml of blood in anticoagulant (EDTA, B-D sterile vacutainer, UK) for blood slides. This was kept refrigerated until use.
2. *Fur clipping*: A small clipping of hair for genetic analyses. Stored in 100% ethanol.

3. *Data on condition*: A record of the fat reserves (thin, lean, optimum, obese, gross) and condition (over or underweight). Each individual was given an overall score on a scale of 1 to 7: '1' for thin to emaciated and underweight, '2' for thin to lean and underweight, '3' for lean and underweight, '4' for lean to optimum, '5' for optimum, '6' for obese and overweight and '7' for grossly overweight.
4. *Diagrams of mange lesion areas*: Dorsal and ventral schematic drawings of mange lesion areas, with and without alopecia, with a record of the predominant stage of the mange infection on a scale from 0-5 (0 none, 1 crusty, 2 not crusty, 4 recovering and 5 unknown).
5. *Clinical Signs*: Notes were also made of any clinical signs consistent with CDV infection as for jackals.

For dead animals, the same *post mortem* protocol and data sheet (Appendices 8.1.3) as those for jackals were used and the same tissues sampled for fixing in formalin and ethanol. The great majority of the dogs sampled from Town Council round-ups could only be sampled after euthanasia; blood was taken from the heart. Where possible the whole carcass was submitted for examination and sampling for histopathology, to the veterinary pathologist, Dr. F. Mettler, at the CVL (Windhoek). Carcasses were only collected in 2002-3 and not in 2001.

#### 2.6.2.1 Ageing of domestic dogs

Dogs were assigned an approximate age in years by the attending veterinarian and classed as under 12 months of age, between 12 and 35 months or  $\geq 36$  months of age. Young dogs of up to 7 months of age were aged by examining the teeth for eruption whilst those over 7 months of age were aged by the tooth wear (Smith 1999) and stature. If only the owner was available the age of the dog was recorded as given and classed accordingly. If neither a veterinarian nor an owner were available, dogs were assigned an age class by project staff and the logistic regression analyses of age class performed with and without these individuals for a comparison of the results.

### 2.6.3 Domestic dog population size

Domestic dog population size can be inferred from human population statistics and estimated human:dog ratios (Perry 1993). Although published human:dog ratios are available for a number of southern and eastern African countries, these were not considered applicable to Namibia as they are site-specific and provided for different subsets of the human population for which there is no equivalent classification in Namibia e.g. by ethnic origin (pers. comm. Arbuckle in Perry, 1993) or by district for which both urban and rural classifications may apply (Brooks 1990). In some cases estimates were only provided for rural areas (Rautenbach *et al.*, 1991, Kitale *et al.*, 2001). Only one publication (Laurenson *et al.*, 1997a) provided data from which a Namibian human:dog ratio could be calculated. But this was not used as the study was carried out using data from 1993 and 1997 and did not include a total count of the human population.

Dog population sizes could also not be estimated from the Town Councils' records. Although the law requires every dog in Namibia to be registered (with a fee payable to the Town Council), this is not enforced. Rather, these figures were used as minimum dog population size estimates.

The dog population estimates for all the coastal towns, the capital city Windhoek, and Namibia as a whole were calculated by applying urban and rural human:dog ratios to human population census data from 2001 (NPC 2003). Urban and rural human:dog ratios were calculated using data extracted from a rabies study of the North Central Division (NCD) of Namibia (Sorin & Mvula 2001) which comprises the following regions: Oshana, Omusati, Ohangwena and Oshikoto. In this study, the domestic dog population was estimated by household questionnaire surveys and a subset of households in three land type categories were surveyed between July and October 2001: urban, rural high density ( $>45$  people/km<sup>2</sup>) and rural low density ( $<45$  people/km<sup>2</sup>). The total numbers of households and the total numbers of dogs belonging to the surveyed households were used to calculate the average numbers of dogs per household for the three land type categories (these data and calculations are detailed in the Appendices, section 8.1.4). Only one value of the average numbers of



dogs per household in the rural category, taken as the midpoint between the rural high density and the low density values, was used as the values in the study by Sorin and Muvla (2001) were seen to be very close in value and no similar classification by density was available from the 2001 human census data.

The study by Sorin and Muvla (2001) did include the total numbers of homes in the three land type categories but these data were obtained from a 1998 database (National Resources Information Service, Namibia) and were therefore underestimates of the numbers of households and population size in 2001. Hence the 2001 human census data was used in the calculations of dog population size.

The numbers of homes in the urban and rural areas of each region in the NCD were calculated from the percentage of the total population in the urban and rural areas and the average household size for that region (NPC 2003). The numbers of dogs for the urban and rural human populations were calculated by multiplying the average number of dogs per household by the numbers of households, in the urban and rural categories. The total number of humans and dogs for the urban and rural areas were totalled and the urban and rural human:dog ratios calculated from these totals (Appendices section 8.1.4).

The estimated urban and rural ratios were then applied to coastal towns' population sizes and the urban and rural population sizes of Namibia as a whole (NPC 2003).

## ***2.7 Histology***

Formalin-fixed tissue samples from jackals (n=10) and dogs (n=4) were routinely processed and embedded in paraffin wax and sections stained by haematoxylin and eosin for histopathological examination by Dr. F. Mettler and Dr. van Verslag at the Central Veterinary Laboratory (Windhoek, Namibia) and PathCare Laboratories (South Africa), respectively. The number of individuals from which tissues could be submitted for testing was limited by financial constraints.

## **2.8 Collection of data by personal communication**

A range of data were obtained from other parties, as listed below:

- a) *Ministry of Environment and Tourism staff*: responsible for the management and patrolling of protected areas as well as the shooting of jackals in poor condition and any dogs which stray into protected areas.
- b) *Ministry of Fisheries and Marine Resources staff*: those responsible for monitoring of the seal harvests and the collection of seal population data.
- c) *Research scientists*: resident in Namibia and working for independent projects or the Ministries of Environment or Fisheries. Those actively involved in long term projects in the coastal region, including the Diamond Area, Lüderitz and the West Coast Recreational Area.
- d) *Veterinarians*: Dr. H. Winterbach, resident in Swakopmund with practices in Swakopmund and Walvis Bay, or the private and state veterinarian (Dr. D. Tinooga) working in Lüderitz, which has no resident veterinarian.
- e) *Tourism guides and hotel staff*: those working in the Lüderitz, the West Coast Recreational Area, particularly those visiting the CCSR and those working at the adjacent hotel.
- f) *NamDeb staff*: those working in the Diamond Area on a daily or weekly basis and responsible for shooting any domestic dogs which enter the area.

Personal communications (pers. comm.) refer to all reports of occurrences of interest which were not observed directly, or confirmed by laboratory testing, by the project. Personal communications include reports of disease cases and outbreaks, encounters between jackals, dogs and brown hyenas, carcasses and historical occurrences of disease in the coastal area. Ministry staff, research scientists and veterinarians are considered 'key informants' in their areas of expertise and the knowledge they provide is, in general, more detailed and reliable than the anecdotal accounts of employees in the tourism industry and other laymen.

Informers working in the study areas were regularly asked to report any carcasses or other occurrences of interest. During the field periods, this contact network was most effective at the northern study sites of Cape Cross and Swakopmund as daily contacts with hotel staff, Ministry officials and weekly encounters with the veterinarian allowed for more accurate and regular reporting than was possible for the Diamond Area and Lüderitz which also more difficult to survey because of the landscape and the limited time spent in the area. Research scientists and Ministry officials, who visited the Diamond Area at least weekly, were interviewed during visits to the area. Seal concessionaires are those responsible for the yearly harvesting of the seals at both study sites and, since they visit the colonies almost daily during harvesting, they were also questioned for information.

## **2.9 Calculation of the SE and 95%**

The angular transformation (Equation 1.0) was applied to the proportion seropositive ( $p$ ) in order to stabilise the variance of the proportion in relation to its mean (Petrie & Watson 1999, Kasuya 2004).

$$\sin^{-1} \sqrt{p} \quad \text{Equation 1.0}$$

This transformation allowed for the approximation of the binomial distribution of the proportion to a normal distribution for the calculation of the standard error (SE).

The standard error (SE) of the percentage positive was calculated using Equation 2.0 where  $p\%$  is the percentage positive (for example, for VN antibodies) and  $n$  is the sample size (Petrie & Watson 1999).

$$SE(p\%) = \sqrt{\frac{p\%(100 - p\%)}{n}} \quad \text{Equation 2.0}$$

Using the angular transformation, the SE for a zero proportion was obtained using Equation 3.0 (Laurenson *et al.*, 1997b), the product of which was multiplied by 100 to obtain the SE for a zero seroprevalence.

$$\left( \sin \left[ 1.96 \sqrt{\frac{821}{n}} \right] \right)^2 / 1.96 \quad \text{Equation 3.0}$$

The upper SE values were plotted on the seroprevalence charts. The SE for proportions were calculated using Equations 2.0 (but substituting  $p\%$  for the proportion and subtracting this from 1 and not 100) and 3.0.

The exact binomial 95% confidence intervals (CI) for seroprevalence values were calculated using the Epi table calculator in Epi Info (Dean *et al.*, 1995). The exact binomial 95% confidence intervals were selected as they are the more conservative (i.e. of larger upper and lower bounds) estimates and cannot result in negative lower bounds for small sample sizes.

## **2.10 Statistical analyses**

### **2.10.1 Logistic regression analyses, seropositivity**

Logistic regression models (SPLUS 2000, MathSoft Inc.) were used to investigate the effects of sampling year, location, age and sex on seropositivity for each disease in jackals and dogs. Seropositivity for each disease was classified as a binary variable (seropositive 1, seronegative 0) and all other variables were classified as categorical. Sampling year was classed as 1, 2 or 3 for 2001, 2002 or 2003 respectively. Location was defined by region for each species. Swakopmund and Walvis Bay were designated as the northern location (location 1) and Lüderitz the southern location (location 2) for domestic dogs. Cape Cross (location 1) and the Diamond Area (location 2) were the northern and southern locations respectively for jackals. Age class was classed as 1, 2 or 3 for <12 months, 12-35 months and  $\geq 36$  months respectively. Male and female individuals were classed as 1 or 2

respectively. The analyses with dog age class as a main effect were repeated with and without dogs sampled in Lüderitz in 2003 ( $n=19$ ) as these were aged by project staff.

For each analysis a generalised linear model (GLM) (Crawley 1993) was fitted to the data using a function with binomial errors, starting with the maximum model including all the terms. In a step-wise deletion any non-significant variables were removed from the model to produce a minimum adequate model. The variables in the minimum model were checked for confounding effects by step-wise removal from the model. Interactions were only tested in models where the main effects were significant, starting with the interactions for all the significant effects and removing the least significant in a step-wise deletion as for the main effects.

The results of the logistic regression analyses of seropositivity are presented in a table format giving the chi-squared value, the degrees of freedom, the residual degrees of freedom and the *P*-value of the full models for each of the significant terms of interest followed by, for the minimum models for each of the terms of interest, the values of the coefficients for the different categories of the variables and the standard error of the coefficients. The value of the coefficient indicates the qualitative change in the probability of an individual being seropositive i.e. if the coefficient is a positive value this indicates an increase in probability with respect to the first category of the variable in question.

### **2.10.2 Analyses for this chapter**

Logistic regression models were used to investigate the effects of trap type and location on the numbers of jackals in each age class and the numbers of males and females sampled; in dogs the effect of location on the numbers of males and females sampled was also investigated using a logistic regression model. For jackals, trap type and location were classed as binary variables and age class and sex as categorical variables; full models were fitted with both age class and sex as explanatory variables and the variable of interest fitted last. Only data from 2002 were used to explore the effect of trap type on age class as this is the only year in

which both trap types were used. For domestic dogs, the effect of location on the numbers of individuals of each sex and age class was investigated using the following models a) sex~age class+location and b) location~sex+age class. The results of these analyses are given in the text as the chi-squared value, the residual degrees of freedom and the *P*-value for the full models.

Due to small sample sizes, a Fisher's exact test was used to test for a difference between locations in the numbers of dogs in each age class.



## 2.11 Results and discussion

### 2.11.1 Jackals

#### 2.11.1.1 Jackal capture and sampling

A total of 87 jackals were captured over the course of 3 field periods (Table 2-3), the majority from Cape Cross. A total of 14 jackals were radio-collared and 62 tagged or fitted with visual collars. Of 11 jackals euthanased, 10 were cases of CDV infection and one was euthanased due to injury (see capture-related injuries, section 2.11.1.3). Serum was obtained from 85 of the captures as two individuals were insufficiently sedated and escaped before the sampling procedure could be completed.

**Table 2-3: A summary of jackal capture by location and year.** Dates of first and last captures, and the total numbers radio-collared, tagged and euthanased for each period.

Year	Location	Dates	<i>n</i>	Radio-collared	Tagged	Euthanased
2001	CCSR	16 to 20 Sept	9			
	CCSR	4 to 10 Nov	3	5	7	0
	Diamond Area	10 to 16 Oct	5	0	5	0
2002	CCSR	7 to 16 Jul	11			
	CCSR	12 Oct to 20 Nov	30	9	23	9
	Diamond Area	6 to 14 Nov	10	0	8	2
2003	CCSR	19 to 31 Oct	19	0	19	0
<i>Total</i>			87	14	62	11

Foothold traps were used at the CCSR and the Diamond Area from October 2002 onwards; only footholds were used in the Diamond Area in 2002. Capture periods in 2001 and the first part of 2002 were limited by the number of traps and the availability of a veterinarian. At Cape Cross, capture at the colony after dawn between July and mid-November was limited by the seal harvest activities.

Table 2-3 excludes two captured individuals which escaped, a re-capture and a pregnant adult captured and released without sedation. One individual managed to

pull the wire off one side of a cage to escape and a second jackal managed to pull its leg free of a foothold.

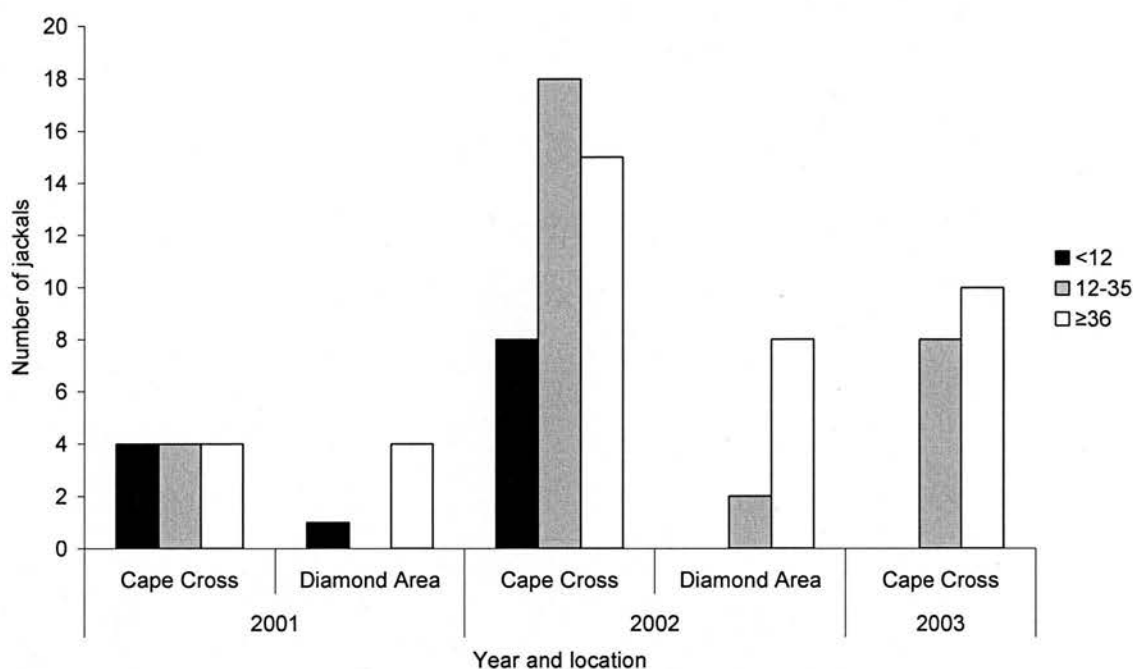
Kelp gulls (*Larus dominicanus*) and brown hyenas were the two non-target species captured in the cages; no non-target species were trapped in the footholds. Two hyenas captured in 2002 in cage traps were released without sampling and two captured in 2003 in foot holds were sampled for serum only. All the hyenas were captured at Cape Cross whilst gulls were captured at both study sites.

#### 2.11.1.2 Jackal age, sex and trap type

A certain degree of error is likely with the use of tooth wear as an indicator of age because of the differences in diet between the jackals in this study and those used by Lombaard (1971). The jackals used to correlate age in years with tooth wear by Lombaard originated from inland areas in South Africa, where they experienced a diet with a much lower sand content than the jackals on the Namibian coast. The application of this methodology to coastal jackals may therefore result in the overestimation of age in years. Therefore, although an estimate of age in years could be obtained for most individuals, jackals were classified into age classes.

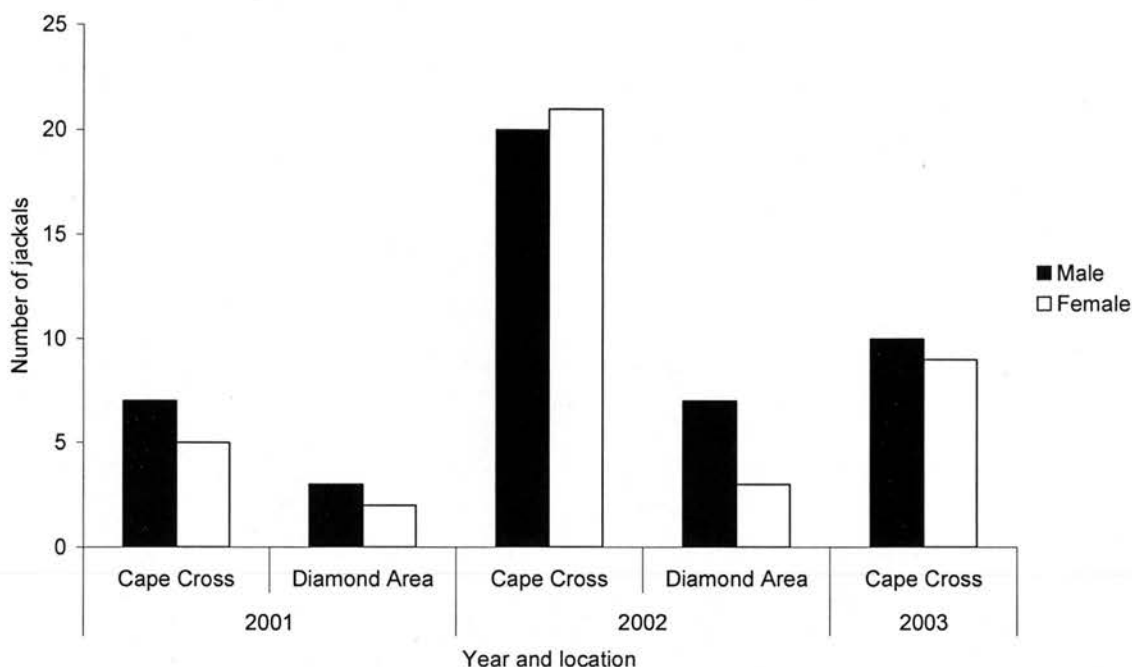
In 2001, the majority of the jackals captured were adults (47%,  $n=17$ ) and very similar numbers of juveniles and sub-adults were captured (29% and 24% respectively). Whilst equal numbers from each age class were captured at Cape Cross in 2001 (Figure 2-6), there were proportionally fewer juvenile and sub-adult individuals captured in the Diamond Area in 2001 and at both sites in 2002 and 2003. The low capture success in the Diamond Area may have contributed to this bias but it persists despite the larger capture effort at Cape Cross and this may be due, at least in part, to the CD epidemic in 2002 (see age-related mortality, Chapter 3, section 3.4.5.3). Due to the low numbers in the juvenile and sub-adult age classes in the Diamond Area, the numbers of individuals in each age class vary significantly between locations, after controlling for sex ( $\chi^2_2=7.84$ , residual d.f.=82,  $P=0.019$ ).

**Figure 2-6: The number of jackals captured in each age class by study site and year.** Excludes one jackal captured in 2003 for which no age classification available. Age classes in months,  $n=86$ .



Overall, similar numbers of males and females were captured with a male to female ratio of 1:0.85 (47 males and 40 females, Figure 2-7). Slightly higher numbers of males were captured during most of the study and this may be due to differences in behaviour between the sexes; female jackals are often more shy and wary than the males (pers. obs. S. Gowtage). Taking into account age class, the numbers of females and males captured did not differ significantly between locations ( $\chi^2_1=1.11$ , residual  $df=82$ ,  $P=0.292$ ).

**Figure 2-7: The number of male and female jackals captured by study site and year.**  
*n*=87.



Overall, similar numbers were captured in the two trap types (Table 2-4) but foothold traps were more successful per capture session than cage traps (Table 2-5); the use of cage traps would have limited capture success. Controlling for age class, the numbers of males and females captured do not vary significantly with trap type ( $\chi^2_1=0.919$ , residual *df*=82, *P*=0.338).

**Table 2-4: The total numbers of jackals captured in cages and footholds for each study site.**

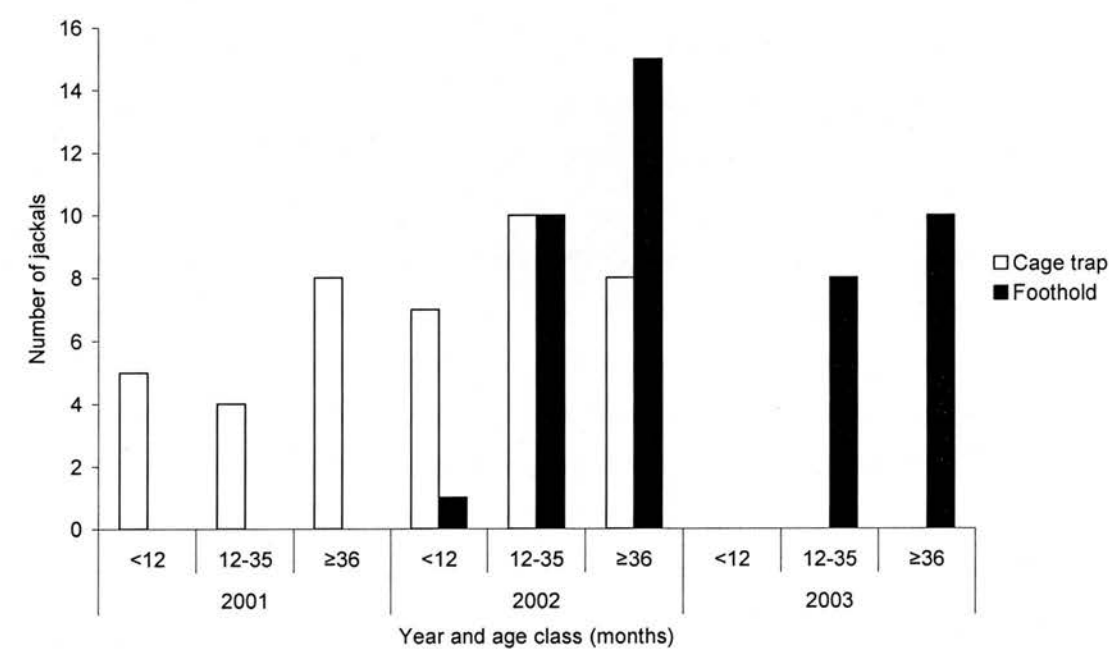
	CCSR	Diamond Area	Total
Cages	37	5	42
Footholds	35	10	45

**Table 2-5: The capture success for foothold and cage traps in the Diamond Area and Cape Cross.** Trap success data (jackals per capture session) was only available for the years and locations listed; units are numbers of jackals per capture session.

		Cages	Footholds	<i>n traps</i>
2001	Cape Cross	1.2	-	4
	Diamond Area	0.5	-	4
2002	Diamond Area	-	1.3	9
2003	Cape Cross	-	2.5	2

Taking sex into account, significantly fewer juveniles and sub-adults were captured in the foothold traps relative to cage traps, in 2002 ( $\chi^2_2=8.66$ , residual d.f.=47,  $P=0.013$ ). This difference may be attributed to a behavioural difference between age classes or to the effects of the CD epidemic. Cage traps did not appear to discriminate between age classes as there was relatively little difference between the numbers of juvenile, sub-adult and adult jackals captured in the cage traps in 2001 and 2002 (Figure 2-8); indeed, there was no difference between age classes by cage trap at Cape Cross in 2001. One would expect the behavioural differences to be evident with cage traps as they, unlike footholds, are not concealed and require the animal to enter an unknown space.

**Figure 2-8: The number of jackals captured in each age class for each trap type and year.  $n=86$ .**



The difference between cages and footholds may be due to increased CDV-related mortality in the younger age classes (see age-related mortality, Chapter 3, section 3.4.5.3). Since foothold traps were used later in the field season in 2002 (i.e. further into the CD epidemic) than cage traps the numbers of juveniles captured would have been lower. A bias in age class or disease-status (the use of bait attracting diseased animals) would affect the age-seroprevalence trends and the seroprevalence estimates, likely overestimating seroprevalence. But one cannot discern if there is a

trap type bias from the available data and without knowing the age structure of the jackal population.

#### *2.11.1.3 Jackal capture-related injuries*

Out of 87 captures, three individuals (3.4%) were affected by capture-related injuries. The first case was an adult female captured in a cage trap the Diamond Area on the 15<sup>th</sup> of October 2001. Examination after sedation with Tiletamine-Zolazepam (70mg) showed the animal to be over eight years of age, in poor condition and possibly pregnant. Mange lesions affected over 70% of the body surface and the teeth of the upper and lower jaws were worn to the extent that feeding would have been affected. This jackal recovered very quickly from the anaesthesia, showing very limited ataxia, and was released from the cage without problems. This individual was found dead on the 24<sup>th</sup> of October, the condition of the carcass indicating that the death occurred approximately 7 days after release. Although the cause of death is unknown, the individual may have been negatively affected by the stress associated with capture and anaesthesia.

The second individual was an adult female captured in a foothold trap in the Diamond Area on the 6<sup>th</sup> of November 2002. This jackal suffered a severe haemorrhagic injury to the front left paw and as a consequence was euthanased. The post-mortem examination by the veterinarian revealed fractures of the distal joints and nail beds of the 3<sup>rd</sup> and 5<sup>th</sup> digits and the absence of the pads and inter-digital skin. The caudal pad was also severely damaged. The trap closed at the level of the metacarpus which showed only a mild haematoma and no fracture. The lesions indicated that the wound was likely self-inflicted after capture.

The third case was a sub-adult female captured on the 9<sup>th</sup> of November in the Diamond Area. The examination revealed that the animal was suffering from a severe respiratory infection and myoclonus of the hind limbs, likely to have been caused by CDV, which resulted in the animal dying during anaesthesia. The jackal



was in poor condition, underweight, and affected by mange. It is unlikely that this individual would have survived long in the wild.

#### 2.11.1.4 *Jackal post mortems*

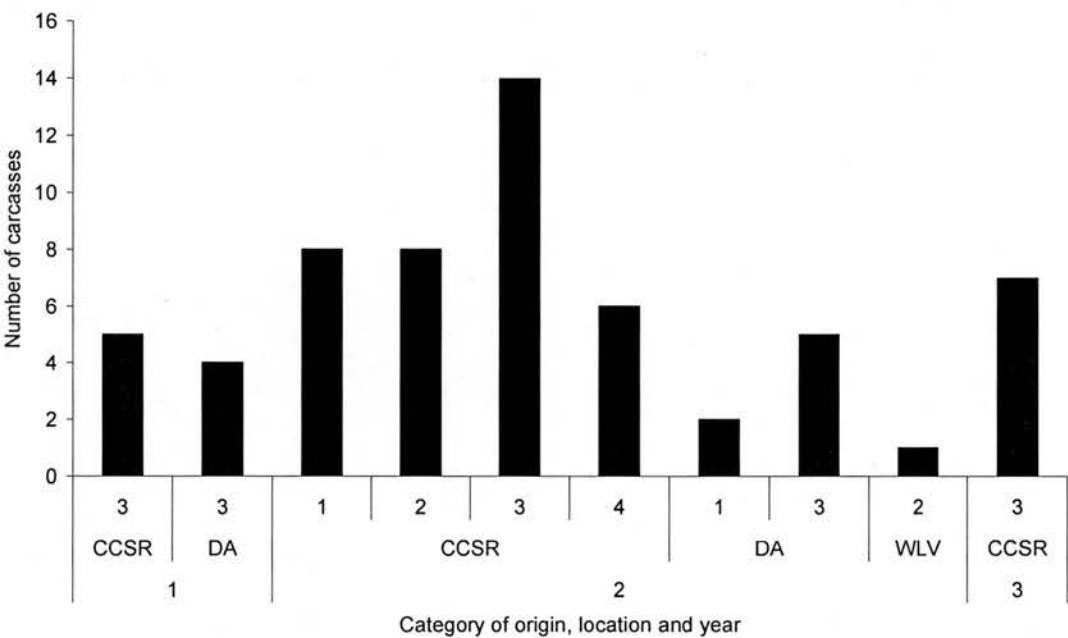
A total of 60 jackal carcasses were encountered during the study period, 48 from Cape Cross, one from Walvis Bay and 11 from the Diamond Area. The carcasses were grouped by origin into four different categories (Table 2-6).

**Table 2-6: The numbers of jackal carcasses from different sources.**

Category	Description	<i>n</i>
1	Animals which were captured and euthanased	10
2	Individuals euthanased in the wild	9
3	Animals found dead	35
4	Carcasses reported but not found	6
Total		60

As most of the carcasses encountered were too decomposed for the collection of diagnostic material, tissues could only be obtained from 23 individuals, most of which were extremely fresh and were suspected CD cases euthanased in 2002 (Figure 2-9). Of these, 12 individuals were sampled for tissues for histopathology which were preserved in formalin. Serum was also obtained from 5 free-ranging individuals from Cape Cross which were euthanased due to CDV infection in 2002.

**Figure 2-9: The numbers of jackal carcasses for each category of origin by location and year.** 'CCSR' Cape Cross Seal Reserve, 'DA' Diamond Area, 'WLV' Walvis Bay. '1, 2, 3' years 2001, 2002 and 2003 respectively. Categories 1-4 as described in Table 2-6,  $n=60$ .



### 2.11.1.5 Jackal population size

A total of 5 inland and 10 coastal distance sampling surveys were performed, yielding a total of 263 sightings from which the distance of 250m was selected as the cut-off distance beyond which marked and un-marked individuals could not be distinguished. A total of 31 coastal mark and re-sight transects were surveyed between the 20<sup>th</sup> of December 2002 and 10<sup>th</sup> of February 2003, 20 of which during the activity period and 11 during the non-activity period; surveys in 2003 were limited by the concurrent sampling of domestic dogs during the CD epidemic in Lüderitz.

Because the average total numbers of sightings (i.e. marked, un-marked and unidentified individuals seen within and outside of the transect area) were considerably lower in the non-activity period (Table 2-7), only activity period sightings (10 surveys of each 5km transect, a total of 20 surveys) were used in the JHE (White 1996a) as this best fulfilled the assumption that all the marked animals are on the area surveyed for each survey.

**Table 2-7: The density of jackals in the coastal transect area, Cape Cross.** As calculated from the average number of sightings of marked and un-marked individuals for the activity and non-activity periods. 'Total sightings' is the number of jackals sighted both within, and outside of, the transect area. Marked and unmarked jackals were only distinguished within the transect area.

Survey period ( <i>n</i> transects)	No. sightings	Total sightings within transect area	Average sightings/ transect	Average density in transect area (jackals per km <sup>2</sup> )
Activity (20)	391	293	14.7	5.9
Non-activity (11)	40	36	3.6	1.4

The density of jackals at the seal colony in this study is considerably lower than that of Hiscocks & Perrin (1988) who calculated a density of  $c.22/\text{km}^2$ . This difference may be due to a larger population size in 1988 and differences in methodology. Of 32 jackals tagged or radio-collared at Cape Cross in 2002, 28 were assumed available for re-sight as 4 individuals were thought to have died of CDV infection in 2002 because they were never sighted again later in 2002 or in 2003. The jackal population of Cape Cross in early 2003 was estimated at 160 individuals (95% CI 129-205) using the JHE (White 1996a) and the numbers of marked and unmarked individuals sighted in the transect area during 20 activity period surveys (Table 2-8).

**Table 2-8: The JHE mark and re-sight jackal population estimate for the CCSR, 2003.** As calculated from the numbers of sightings of marked and un-marked jackals from 20 surveys of the coastal transect during the activity period.

Category of individuals	Numbers of sightings	JHE estimate	95% CI
Marked	51	160	129-205
Un-marked	242		
Total	293		

A number of the assumptions of the JHE may have been violated in this study leading to a bias in the population estimate. Jackals have been known to lose their ear-tags which is why most animals in this study were fitted with two tags as to ensure that most would still be identified as captured. Visibility biases were kept to a minimum by only surveying on days when any early morning fog had cleared. Observations during radio-tracking indicate that young jackals can form loose social groups and groups will also form when feeding on carcasses therefore violating the

assumption of independence some of the time. Neal *et al.*, (1993) indicate that the bias caused by an unequal sighting probability for each individual is small ( $\pm 8\%$ ) although it does result in lower coverage of the 95% CI and a decrease in precision. Considering the precautions taken to avoid violating the assumptions of the JHE in this study and the narrow width of the confidence intervals of the population estimate, the population estimate for Cape Cross was considered reasonable and useful.

A seal pup:jackal ratio of 234:1 (range 290:1-182:1) was obtained from the 2001 point estimate of the seal pup population ( $n=37,394$ , (Mukapuli 2004)) and the JHE estimate of the jackal population of Cape Cross (Table 2-9). This ratio was then extrapolated to the Diamond Area study site and the coast as a whole (Table 2-9).

**Table 2-9: The estimates of the jackal population for the study sites and the coast.** As calculated by extrapolating the seal pup:jackal ratio for CCSR to the seal population estimates of the Diamond Area and the coast as a whole.

Location	Seal pup population estimates <sup>1</sup>	Ratio seal pups:jackals (95% CI)	Estimates of the jackal population size (95% CI)
CCSR	37,394	234:1 (290:1-182:1)	160 (129-205)
Diamond Area	33,377		142 (115-183)
Namibian coast	113,101		483 (390-621)

<sup>1</sup>(Mukapuli 2004).

The estimate of the jackal population at Cape Cross, and by extension, estimates derived from the Cape Cross pup:jackal ratio, are potentially affected by a number of factors. Firstly, the use of cage traps which were less efficient than footholds, may have limited the fraction of the population which was eartagged and therefore increased the confidence intervals surrounding the population estimate. Secondly, the use of a seal pup:jackal ratio excludes those jackals present at the mainland non-breeding colonies and those supported by the large bird populations of the coast; this would underestimate the jackal population. Thirdly, there is unknown error in the aerial census estimates of the pup population as the census does not include those which are not visible, those not born by the census date (mid-December), and those

which have died by the census date (Pallett 2000); mortality in the first 30 days of life is considerable and estimated at 20% (De Villiers & Roux 1992). An underestimate of the pup population would overestimate the jackal population. Nonetheless, an approximation of the coastal jackal population size is useful when considering the maintenance of pathogens in this population.

## 2.11.2 Domestic dogs

### 2.11.2.1 Domestic dog age and sex

Of the 100 individuals included in this study, 85 were assigned to an age class (Table 2-10 and Figure 2-10) of which 26 from Lüderitz, sampled in January 2003, were aged by project staff and not a veterinarian. Of these 26, serum was obtained from 19 individuals. The logistic regression analyses in Chapters 3 and 5 were repeated with and without these 19 individuals. There was no difference between locations in the numbers of dogs in each age class (Fisher's exact test:  $P=0.816$ ).

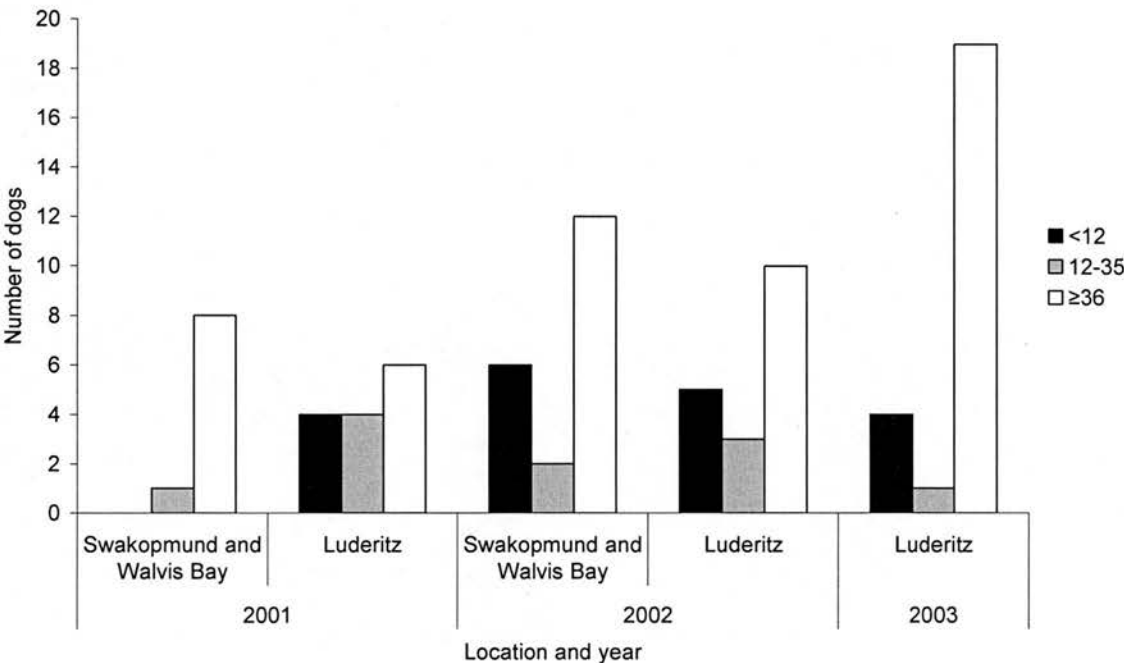
**Table 2-10: The percentages of dogs in the three age classes.** Age classes in months.  $n=85$ .

Age class	Percentage of dogs (%)
<12	22.4
12-35	12.9
$\geq 36$	64.7

One would expect the higher proportion of dogs in the youngest age class as dog populations from developing communities typically have a very high turnover and short life expectancy (Brooks 1990, Laurenson *et al.*, 1997a, Butler & Bingham 2000). The distribution of dogs in the age classes in this study may have been affected by a) the inaccuracies associated with ageing dogs over 6-7 months of age from the patterns of tooth wear as the rate and pattern of tooth wear vary greatly due to differences in diet, environment and behaviour (Smith 1999), and b) the sources of the dogs which may not have allowed equal access to different age classes in the population e.g. Town council round-ups may omit very young dogs which may not

stray from the household premises. Ages obtained from owners are also subject to error (Rautenbach *et al.*, 1991). But in the absence of the analysis of cementum layers the methodologies used in this study were the only practical alternatives.

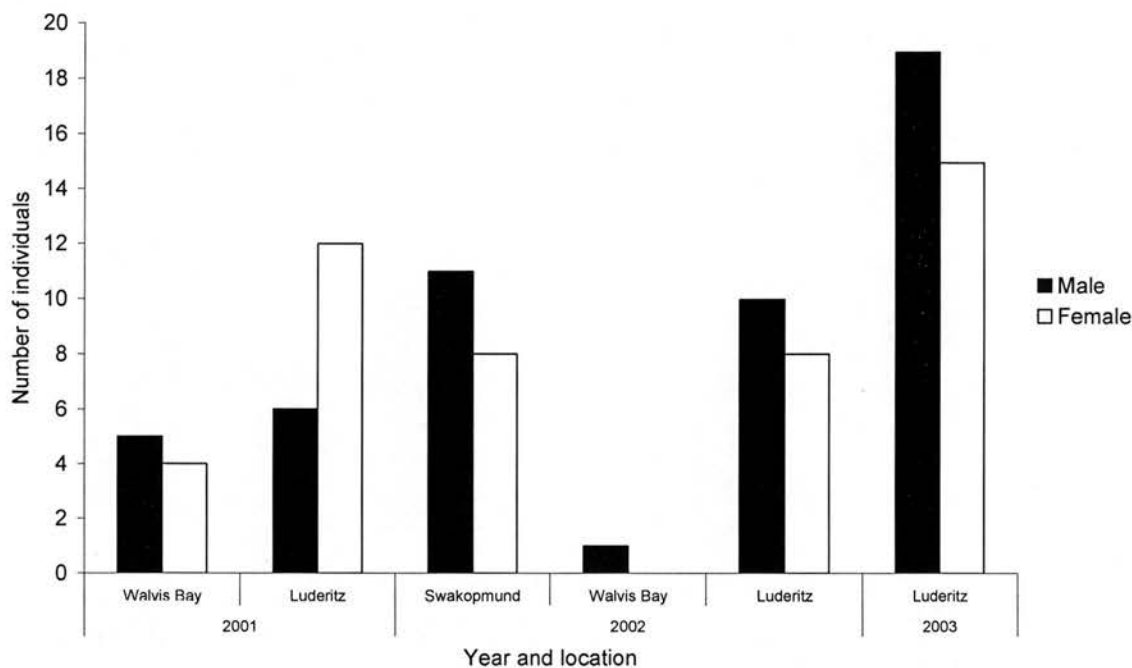
**Figure 2-10: The number of dogs in each age class by study site and year.** Age classes in months. *n*=85.



Sex was recorded for 99 of 100 domestic dogs included in this study, of which 52 were male and 47 were female resulting in a male:female ratio of 1:0.9. For most locations, except Lüderitz in 2001, the number of males sampled was higher than the number of females (Figure 2-11). Controlling for age class, there was no significant difference between the numbers of males and females sampled between locations ( $\chi^2_2=1.88$ , residual d.f.=79,  $P=0.392$ ).



**Figure 2-11: The numbers of male and female dogs by location and year.  $n=99$ .**



**2.11.2.2 Domestic dog serum and tissue sampling**

A summary of the number of serum samples and their origin is presented in Tables 2-11 and 2-12. The dogs collected by the Town council round ups and those sampled in Swakopmund (at the rubbish dump and resettlement community) and Lüderitz (rabies vaccination campaign) were ‘neighbourhood dogs’ as defined by Perry (1993): “a proportion of such dogs’ essential needs are provided intentionally by humans and they are semi-restricted or completely free, having free access to the rest of the population some or all of the time respectively”. The dogs visiting the veterinary clinics were classed as fully dependent ‘restricted dogs’ but most of these are able to contact other un-restricted individuals through fences and other incomplete barriers, as observed when sampling dogs in the towns (pers. obs. S. Gowtage).

**Table 2-11: The numbers of dogs sampled for serum and their origins by month for each location, 2001 to 2003.** Sources of dogs: Town Council round-ups, vet clinic, Swakopmund rubbish dump and resettlement community and the rabies vaccination campaign in Lüderitz.

Location	Date	Sources of dog sampled				Total
		Town council	Vet clinic	Swakopmund	Rabies vaccination	
Lüderitz	Mar-01	13	0	0	0	13
	Oct-01	5	0	0	0	5
	Oct-02	0	5	0	0	5
	Nov-02	13	0	0	0	13
	Jan-03	25	0	0	4	29
Walvis Bay	Aug-01	9	0	0	0	9
Swakopmund	May-02	0	1	0	0	1
	Nov-02	0	1	15	0	16
	Dec-02	0	1	0	0	1
Total		65	8	15	4	92

Tissues in both ethanol and formalin were obtained from 6 individuals (Table 2-12). One of these was found dead and is thought to have died of CDV infection and the remaining 5 were euthanased due to severe signs of CDV infection.

**Table 2-12: Domestic dog tissue samples, 2002 and 2003.**

Year	Location	Sources of carcasses	<i>n</i>
2002	Swakopmund	Vet clinic	2
	Walvis Bay	Vet clinic	1
2003	Lüderitz	Town council	2
		Rabies vaccination	1
Total			6

### 2.11.2.3 Domestic dog population size

The minimum dog population sizes for Swakopmund and Lüderitz, obtained from the Town Council records were 2695 and 7 individuals respectively. These are likely to be underestimates of the actual dog populations due to the fact that registration is not enforced. A guestimate of the Lüderitz dog population was approximately 700 individuals (pers. comm. I. Wiesel, research scientist).

The urban and rural human:dog ratios were calculated to be 6.6:1 and 4.0:1 respectively (Appendices, Chapter 2, section 8.1.4). Bishop (Bishop 2001) provides a comparable human:dog ratio of 6.2:1 for the urban locality of Marburg in South Africa.

To obtain dog population estimates for 2002, the regional and national human growth rates (NPC 2003) were applied to the 2001 census totals for the towns and the urban and rural population census totals of Namibia respectively (Table 2-13). The appropriate urban or rural ratio was then applied to the estimated human population size for 2002. The total coastal dog population for 2002 was estimated at 13,576 and the total Namibian dog population at 408,487 (Table 2-13).

**Table 2-13: The dog population estimates for each of the coastal towns and the rural and urban human populations of Namibia, 2002.**

Location or population	Human population 2001	Human growth rate (%)	Estimated Human population 2002	Human:dog ratio applied	Estimated dog population
Walvis Bay	43,611	1.3	44,178	6.6:1	6,694
Swakopmund	23,808	1.3	24,118	6.6:1	3,654
Lüderitz	13,295	1.3	13,468	6.6:1	2,041
Oranjemund	4,451	1.3	4,509	6.6:1	683
Henties Bay	3,285	1.3	3,328	6.6:1	504
<i>Total coastal</i>	<i>90,750</i>		<i>89,600</i>		<i>13,576</i>
Windhoek	233,529	4.0	242,870	6.6:1	36,799
Namibian urban	603,612	2.6	619,306	6.6:1	93,834
Namibia rural	1,226,718	2.6	1,258,613	4.0:1	314,653
<i>Total Namibian</i>	<i>1,830,330</i>		<i>1,877,919</i>		<i>408,487</i>

#### 2.11.2.4 Summary of sampling for domestic dogs and jackals

A summary of the sampling performed during the field periods is given in Table 2-14 below.

**Table 2-14: The numbers of jackal and dog serum and tissue samples collected for each year location.**

Year	Location	Species	Serum samples	Tissue samples
2001	Cape Cross	Jackals	12	0
	Wolf and Atlas	Jackals	5	0
	Bays			
	Swakopmund and Walvis Bay	Domestic dogs	10	0
	Lüderitz	Domestic dogs	17	0
2002	Cape Cross	Jackals	44	10
	Wolf and Atlas	Jackals	10	
	Bays			
	Swakopmund and Walvis Bay	Domestic dogs	15	3
	Lüderitz	Domestic dogs	21	2
2003	Cape Cross	Jackals	19	0
	Wolf and Atlas	Jackals	0	0
	Bays			
	Swakopmund	Domestic dogs	0	0
	Lüderitz	Domestic dogs	29	3
Totals		Jackals	90	12
		Domestic dogs	92	6

### **Chapter 3: Canine distemper in jackals and dogs**

### **3.1 Abstract**

A number of high-profile CD epidemics in terrestrial and aquatic carnivores have raised concerns about the role that domestic dog populations play in the transmission, maintenance and spill-over of this pathogen to wild species, but the role played by wild canids has been largely overlooked. Black-backed jackals are widespread in sub-Saharan Africa and have been indirectly implicated in CD epidemics. This study investigated the transmission of CDV between black-backed jackals and domestic dogs on the Namibian coast to determine if CDV was persisting in either of these species and to assess the risk of spill-over to sympatric Cape fur seals and brown hyenas. The patterns of exposure and infection were examined in both jackals and dogs using serology, case reports, histology and immunocytochemistry. In 2001, seropositive dogs were detected but no seropositive jackals were found. In early 2002 a CD epidemic occurred in the domestic dog population of the capital city, Windhoek. This then spread to affect the domestic dogs of the coastal towns and subsequently spilled-over to jackals at the seal colonies along the length of the Namibian coast. The naivety of the jackal population is confirmed by the absence of any age-specific morbidity, the relatively high mortality (*c.* 7-16%) as compared to 2001, and the high seroprevalence (74.1%, 95% CI 60.3-85.0) attained during the epidemic. Seroprevalence increased with age in jackals and this is likely due to age-specific mortality.  $R_0$  for CDV in jackals was estimated to be between 1.1 and 1.9. The demographic and ecological characteristics of the jackal population preclude the maintenance of CDV in this population. The domestic dogs of the coastal and inland towns may act as a maintenance population, and the jackals as effective hosts in the chain of transmission. Jackals augmented the epidemic spread of CDV, resulting in spill-back into the dog population and the possible exposure of brown hyenas and Cape fur seals. The role of the jackals in the spread of CDV has important implications for other wildlife species of conservation interest which may be susceptible to CDV, both on the coast and in other areas of Namibia where jackals are prevalent.



### 3.2 Introduction

The involvement of jackals in the transmission of CDV and their potential role as reservoirs has been inferred from a number of serological surveys and descriptions of population declines associated with CDV (Table 3-1). But in the absence of any described epidemics of CD in jackals this information alone is not sufficient to discern the role of jackals in the epidemiology of CD, be they reservoirs, hosts in the chain of transmission linking different populations, or simply victims of spill-over.

The scarcity of knowledge is due in part to the difficulties associated with exploring the complex dynamics of multi-host viral pathogens. The capture and sampling of wild animals to collect blood and tissue samples is costly and labour-intensive. Capture success for low-density, 'wary' species may be low and access to infected carcasses which are not too decomposed or scavenged for the retrieval of diagnostic material is very limited. The preservation of blood or tissue samples in the field poses additional difficulties with limited access to suitable fridge and freezer facilities. For example, tissues for virus isolation often require freezing in liquid nitrogen which necessitates specialist equipment and supplies not often available in remote field areas. An easier option, and one which has yielded a wealth of data, is that of serology – the detection of exposure to a pathogen by testing for antibodies in the serum. Although this type of sampling has its limitations, in that it provides evidence of exposure and not actual infection and the deceased cannot contribute to the sample group, it can be of great value if related to disease-induced mortality and age data of the sample group.

Exposure to CDV varies greatly between jackal species and country (Table 3-1) but the studies are consistent in terms of the potential role of the jackal species as reservoirs or effective links for disease transmission between domestic and wild species: "*jackals.....could serve as an important link in disease transmission between domestic animals and wild carnivores*" (Alexander *et al.*, 1994), and Sharmir *et al.* suggest that "*.....jackals may serve as an important reservoir and a potentially efficient transmitter of certain canine pathogens*" (Sharmir *et al.*, 2001).

This is due to the jackals' widespread distribution in both human and wildlife habitats throughout sub-Saharan Africa, their susceptibility to a wide range of pathogens commonly found in domestic dogs, and their role as opportunistic carnivores and scavengers which brings them into contact with a wide range of wild and domestic species (Wyman 1967, Alexander *et al.*, 1994, Spencer *et al.*, 1999, Macdonald 2001, Sharmir *et al.*, 2001).

**Table 3-1: A summary of the exposure to, and suspected occurrences of, CDV in jackals.** Black-backed jackal (BB): *Canis mesomelas*, Side-striped jackal (SS): *C. adustus*, Golden or Asiatic jackal (GJ): *C. aureus*. Percentages are seroprevalences.

Species	Study type	Year(s)	Location	Data
BB	Socioecology <sup>1</sup>	1978-9	Tanzania, Serengeti	Population decline: decrease in sightings
BB GJ	Unpublished data <sup>2</sup>	1980-81	Masai-Mara, Kenya	Population decline: decrease in sightings
BB	Case reports <sup>3</sup>	1985	Outjo, Namibia	-
BB SS GJ	Pathogen survey <sup>4</sup>	1987-88	Various locations, Kenya	9.0% (n=55) 33.3% (n=3) 0.0% (n=16)
BB SS	Pathogen survey <sup>5</sup>	1990-3	Various locations, Zimbabwe	63.6% (n=14) 50.0% (n=8)
BB	Epidemic <sup>6</sup>	1991	Masai-Mara, Kenya	Population decline: decrease in sightings
BB GJ	Epidemic <sup>1</sup>	1994	Tanzania, Serengeti	Morbidity observed (n=3)
GJ	Pathogen survey <sup>7</sup>	1998	Various locations, Israel	52.2% (n=46)

References: 1 Roelke-Parker *et al.*, (1996); 2 Alexander *et al.*, (1995); 3 Schneider (1994); 4 Alexander *et al.*, (1994); 5 Spencer *et al.*, (1999); 6 Alexander & Appel (1994); 7 Sharmir *et al.*, (2001).

Aside from basic serological surveys, in which the data is not analysed in conjunction with age and mortality data, our knowledge of the jackals' involvement in confirmed CDV wildlife epidemics and the impact of CDV on this species, is

limited to observed population declines or observations of clinical signs in small numbers of animals, in conjunction with outbreaks in other species (Moehlman 1983, Alexander *et al.*, 1994, Roelke-Parker *et al.*, 1996). In the case of the study by Moehlman (1983), it is not clear how the population decline was attributed to CDV (Roelke-Parker *et al.*, 1996), on the basis of un-specified clinical signs and in the absence of any form of testing.

The limited knowledge of the role played by jackals in the transmission of generalist canid pathogens is also due to the slow accumulation of evidence for exposure to diseases other than CDV as the majority of the epidemiological research focuses on species of conservation interest. To date there have been no long-term CDV surveillance programs for jackals which would provide the necessary data to address the 'reservoir' question. Longitudinal serological studies, such as the 10-year study of the lions in the Serengeti (Packer *et al.*, 1999), which can provide temporal patterns of exposure and age-seroprevalence curves for a number of pathogens, are required to establish whether a pathogen is endemic or epidemic in a population. Such data is lacking for jackals.

As an example, the study of the 1994 CD epidemic in the Serengeti lions (Roelke-Parker *et al.*, 1996) highlighted the potential role played by wild canids in the transmission of CDV and the urgent need to include wild canids such as jackals and bat-eared foxes in disease surveillance programs. Unconfirmed fatal epidemics are thought to have occurred in Serengeti jackals between 1977 and 1979 (Moehlman 1983, Roelke-Parker *et al.*, 1996) and the retrospective analysis of lion sera from the region reveals exposure to CDV prior to the 1994 epidemic (Roelke-Parker *et al.*, 1996). The opportunistic surveillance conducted as part of studies of other species such as spotted hyenas (*Crocuta crocuta*) do not provide sufficient information (Haas *et al.*, 1996).

Serological, demographic and genetic analyses (Harder *et al.*, 1995, Roelke-Parker *et al.*, 1996, Carpenter *et al.*, 1998, Cleaveland *et al.*, 2000) indicate that the epidemic in lions was a result of spill-over from domestic dogs living outside the Serengeti

National Park. But it is possible that lions were also infected directly from wildlife, as species other than lions may have been infected by domestic dogs (Cleaveland *et al.*, 2000). Inter-species transmission, which for CDV requires only close contact, is likely to be a frequent occurrence (Alexander *et al.*, 1994, Harder *et al.*, 1996, Murray *et al.*, 1999). In addition to the three suspected cases in jackals (Table 3-1), CDV-induced disease was confirmed in spotted hyenas (Haas *et al.*, 1996) and in two bat-eared foxes (Roelke-Parker *et al.*, 1996). But in the case of the bat-eared foxes, it is not known how this was confirmed and the scale of the mortality was not documented for any of these species.

It is thought that CDV may now be persisting in the Serengeti system independently of spill-over from the domestic dog populations, but the role of the different wildlife species is as yet unknown (pers. comm. Dr. S. Cleaveland). Circulating virus has been detected from seropositivity in young lions (>18 months of age), since the 1994 epidemic, in the absence of CDV-induced morbidity and mortality and the majority of villages surrounding the park from which domestic dogs have been sampled have since resulted seronegative (pers. comm. Dr. S. Cleaveland).

CD epidemics in the Masai Mara ecosystem in Kenya apparently followed a similar pattern to the 1994 Serengeti outbreak. But once again, the involvement of other wildlife species is unknown although it is thought that wildlife were responsible for the spread of the Serengeti outbreak to the Masai-Mara National Reserve (Kock *et al.*, 1998). Between 1990 and 1991 a CD epidemic occurred among domestic dogs living near the Masai Mara National Reserve, resulting in increased mortality and concurrent decreases in populations of jackals (*Canis* spp.), bat eared foxes and African wild dogs (Alexander & Appel 1994). Spotted hyenas in the reserve also showed an increased exposure to CDV between 1990 and 1992 (Alexander *et al.*, 1995).

In support of the hypothesis that wildlife may also act as a source of CDV, the results of a serosurvey of spotted hyenas (Harrison *et al.*, 2004) in the Masai-Mara National Reserve between 1993 and 2001, suggested that this species may play a role in the

ecology of CDV in this area but that it does not act as a reservoir for CDV. The proximity to human habitation actually decreased the risk of exposure to CDV relative to hyenas further from human habitation and although the serology provided only a measure of exposure to CDV, the variation in seroprevalence in the younger age classes indicated that the dynamics of CDV are constantly changing. More definite conclusions require evidence of actual infection and CDV-induced mortality in this study's host population.

In Namibia, in an extensive but not exhaustive review, spanning 150 years, of the occurrence and epidemiology of domestic animal diseases (Schneider 1994), CD was recorded as a disease of domestic dogs alone, as the overwhelming majority of the cases occurred in this species. Although CD was "prevalent in all parts of Namibia" it was considered to be "of lesser importance" because of extensive vaccination but still "very prevalent in the dog populations of townships and squatter areas". Control of CD by vaccination means it was ranked as the 3<sup>rd</sup>, and not 1<sup>st</sup>, most important disease of domestic dogs but this may only be applicable to the dogs of developed communities. Dogs in developing communities have very low levels of vaccination coverage (Leisewitz *et al.*, 2001). The description of the occurrence of CD cases in wildlife, although acknowledged as a growing area of interest, was limited to two references in a nine-page table of diseases of game species in which rabies and anthrax predominated. Of the two references, one was of CD in a black-backed jackal (Table 3-1) and the other in an aardwolf in the Windhoek area in 1987; there was no information provided on sample size or test methodology and the references for these cases were unpublished annual reports of the Division of Veterinary Services (Windhoek).

More recently, exposure to CDV in Namibia has been documented in African wild dogs (Laurenson *et al.*, 1997b) as well as cheetahs (*Acinonyx jubatus*) (Munson *et al.*, 2004) with seroprevalences of 66.7% (n=6) and 24.3% (n=70) respectively. Both species in these studies are sympatric with domestic dogs which may be harbouring the virus (Tsumkwe district, seroprevalence 44.3%, n=70, Laurenson *et al.*, 1997), but the sources of the infection and the viral strain(s) have yet to be investigated.

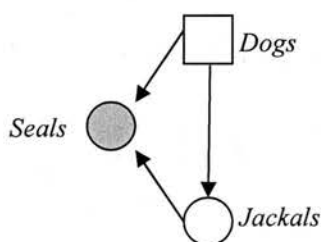
Since antibodies to CDV were detected in cheetahs of all age classes between 1995 and 1998, Munson *et al.* (2004) suggest that a CD epidemic was occurring in Namibian cheetahs, concurrent with other outbreaks in other parts of sub-Saharan Africa (Alexander *et al.*, 1996). But such a conclusion must be considered in the light of the study only being a record of exposure and that no further evidence was provided to link this with epidemics elsewhere.



### 3.3 Aims

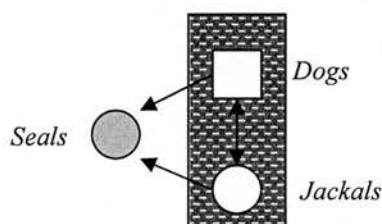
A framework for the investigation of potential reservoir species (Haydon *et al.*, 2002), reviewed in Chapter 1, can be used to formulate hypotheses for the epidemiology and maintenance of CDV in this study system. Under the first hypothesis (Figure 3-1) jackals are ‘victims’ of spill-over from domestic dogs. In this scenario jackals would not be able to maintain the virus but act as a source of CDV and transmit the disease to the seals. The domestic dogs are the maintenance population and may also transmit CDV directly to the seals.

**Figure 3-1: Hypothesis 1- Jackals as the ‘victims’ of spill-over from domestic dogs.** Squares indicate a maintenance population, blank circles a source population and filled circles a target population.



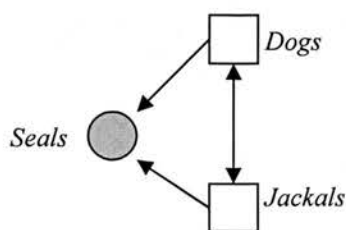
In the second hypothesis (Figure 3-2) jackals are part of a maintenance community with domestic dogs, and could transmit the disease to both seals and dogs.

**Figure 3-2: Hypothesis 2- Jackals form part of a maintenance community with domestic dogs for the transmission of CDV to seals.** The shaded/dashed area indicates a maintenance community comprising jackals and dogs; other symbols as in Figure 3-1.



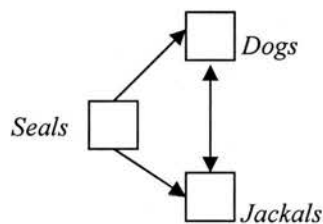
The third hypothesis is that jackals act as an independent maintenance population for CDV (Figure 3-3); they may or may not be part of a maintenance community with domestic dogs.

**Figure 3-3: Hypothesis 3- Jackals may act as an independent reservoir for CDV, transmitting the virus to seals and dogs.**



Of course it is also possible that the seals may also play a maintenance role in this system and this is discussed further in Chapter 4. In one hypothesis, as shown in Figure 3-4, the large seal population would act as a maintenance species for CDV and transmit it directly to jackals or domestic dogs.

**Figure 3-4: Hypothesis 4- The Cape fur seals may act as a maintenance population for the transmission of CDV to jackals and dogs, also capable of maintaining CDV.**



The aim of this study is to investigate the above hypotheses (Figures 3-1. 3-2, 3-3) using spatio-temporal patterns of disease, case descriptions of clinical signs, histopathological and serological data. This chapter presents an investigation of the first reported outbreak of CDV in jackals and presents evidence from which the role played by jackals in this system is inferred.

## **3.4 Materials and methods**

### **3.4.1 Reporting of cases**

As described in detail in Chapter 2 (section 2.8), reports of cases were obtained from discussions with a variety of informants including research scientists, veterinarians, Ministry of Fisheries and Ministry of Environment staff, and locals working in the tourism industry and seal concessionaries (those responsible for the harvesting of the Cape Fur Seals). In each case, details such as location, number and condition of the animal or carcase(s) were recorded. This data was used to assist the reconstruction of the spatio-temporal pattern of the CD epidemic.

### **3.4.2 Sample collection**

Details of the protocols for the collection and processing of serum and tissue samples are described in Chapter 2 and a summary of the sampling is provided in section 3.3.3. Serum samples were obtained from 90 jackals and 92 dogs. For each individual, data on condition and the presence and absence of clinical signs of CDV infection were also collected, in addition to age and sex data.

In summary, jackals in the Diamond Area (Wolf and Atlas Bay) and the CCSR were captured using cage and foothold traps over 7 capture periods between 2001 and 2003. Serum was obtained from 85 of the 87 captures and from an additional 5 individuals euthanased (due to severe CDV infection) in the field. Tissues for histology were obtained from 12 individuals.

Sera were obtained from 92 of the 100 dogs included in this study, from all three of the largest coastal towns. The majority originated from Town Council round-ups of unclaimed neighbourhood dogs, before during and after the CD epidemic. Tissues were obtained from 6 individuals which were either euthanased because of CDV infection or likely died from CD.

Tissues for histology were submitted to the veterinary pathologist, Dr. F. Mettler, at the CVL, Windhoek and to PathCare, South Africa.

### 3.4.3 Summary of sampling

A more detailed breakdown of the sampling detailed in Table 2-14 (page 81) is given in this section. Tables 3-2, 3-3 and 3-4 give the numbers of jackals sampled for serum and tissues during the study period. The carcass categories for jackals are as follows: those captured and euthanased, those euthanased whilst free-ranging, those found and those reported but not recovered.

**Table 3-2: The numbers of jackals sampled for serum and tissues by study site, 2001.** *n*=26.

Location	Capture	Carcasses found	<i>Serum</i>
CCSR	12	5	12
Diamond Area	5	4	5
Total	17	9	17

**Table 3-3: The numbers of jackal captures and carcasses sampled for serum by study site, 2002.** \*Excludes a case of self-mutilation euthanased in the Diamond Area; 'CS' clinical signs. *n*=85.

Location	Captures		Carcasses				<i>Serum</i>
	Capture	Released with CS	<i>Captures euth.</i>	<i>Free-ranging</i>	<i>Found</i>	<i>Reported</i>	
CCSR	41	8	8	8	14	6	44
Walvis Bay	0	0	0	1	0	0	0
Diamond Area	10	2	1	0	5	0	10
Total	51	10	9*	9	19	6	54

**Table 3-4: The numbers of jackals sampled for serum from the CCSR, 2003.** There is no data available for carcasses found in October 2003. Jackals *n*=20, brown hyenas *n*=2.

Species	Capture	Re-capture	<i>Serum</i>
Jackals	19	1	20
Brown hyenas	2	0	2
Total	21	1	22

Tables 3-5, 3-6 and 3-7 give the numbers of domestic dogs sampled for serum and tissues over the course of the study period. A number individuals in Lüderitz in 2003 could only be sampled for data and not serum or tissues.

**Table 3-5: The numbers of domestic dogs sampled for serum, 2001.** No carcasses were recovered from the town council-round-ups.  $n=27$ .

Location	Serum
Walvis Bay	9
Lüderitz	18
Total	27

**Table 3-6: The numbers of domestic dogs sampled for serum and tissues, 2002.**  $n=38$ .

Location	Serum	Carcasses
Swakopmund	18	2
Walvis Bay	0	1
Lüderitz	18	0
Total	36	3

**Table 3-7: The numbers of domestic dogs sampled for serum and tissues from Lüderitz, 2003.**  $n=35$ .

Source	Serum	Carcasses	Data
Town council round-up	25	0	1
SPCA kennels	0	1	0
Rabies vaccination campaign	4	2	3
Total	29	3	4

### 3.4.4 Immunocytochemistry

The procedure was performed by Mrs. J. Chesher at Intervet Laboratories (UK). Financial and laboratory constraints limited the testing to one dog and one jackal. Ethanol-fixed tissues from one jackal and one dog were stained for CDV antigen (Catelli *et al.*, 1998) using goat anti rabbit serum in a peroxidase-anti-peroxidase (PAP) reaction. Briefly, the tissues were de-waxed utilising xylene and industrial methylated spirit (IMS). Any existing peroxidase was blocked with  $H_2O_2$  in IMS and after washing in TRIS buffered saline solution (TBS) the tissues were incubated with

normal goat serum (blocking agent, 1:40 dilution). Excess goat serum was removed and the rabbit anti-CDV polyclonal antibody added and incubated. The goat anti-rabbit serum was added and after incubation, the tissues were washed in TBS and acetate/citrate buffer and placed in nickel enhanced diaminobenzine (DAB) substrate. The reaction was stopped by washing in TBS and the tissues were counterstained with Light Green stain, then dehydrated in IMS, cleared in xylene and then mounted. Urinary bladder and small intestine tissues from a known CDV positive dog and a known negative dog were included as controls. Sequential sections of the jackal and dog tissues incubated with normal rabbit serum were used as test controls.

### **3.4.5 Rabies testing**

Refrigerated whole brains from jackals and dogs were submitted directly, or via a state veterinarian, to the Central Veterinary Laboratory (Windhoek) for testing by Fluorescence-Antibody Test (FAT) (Bourhy & Sureau 1990).

### **3.4.6 Estimating the month of death**

For jackals which were found dead in the field, it was possible to estimate the time of death from the condition of the carcass and in some cases, from reports by research scientists and other informants on the presence and absence of carcasses in particular locations.

As described in Chapter 2 (section 2.5.2.4), carcasses were categorised according to their condition (Table 3-8). For carcasses categorised as extremely fresh or slightly decomposed, the time of death could be estimated at  $\leq 24$ hrs prior to discovery (Table 3-8). Those carcasses which were moderately decomposed were classed as having died up to 48hrs ago. The carcasses in an advanced state of decomposition were likely to have been dead for 2-7 days. The category of 'advanced decomposition' includes those which died in the month they were found and which were

differentiated from those which died the same week by the absence of any recognisable tissue and the presence of moisture, decaying tissue and numerous insects and possibly maggots. The state of carcasses classed as 'indeterminate' varied considerably, and therefore a month of death could not be assigned to carcasses in this category. But if the carcass was in reasonably good condition in that all the teeth were still present and the individual could be aged, it was assumed that the individual had died in the year it was found (unless found in January or February in which case it was assumed to have died the previous year).

The time of death was estimated to the nearest month for all carcasses except those in the indeterminate category for which no further information, in addition to condition, was available. The estimates for each category were supported by observations from carcasses which occurred in amongst the seals and which could not be recovered and advice from the veterinarian (Dr. H. Winterbach).

**Table 3-8: Estimating the time of death for jackal carcasses found in the field.**

Category	Condition	Estimated time of death
Extremely fresh	As if just died, no bloating	≤24hrs
Slightly decomposed	Slight bloating, blood imbibition visible	≤24hrs
Moderately decomposed	Moderate bloating, skin peeling, organs beginning to deteriorate but still recognisable	24-48hrs
Advanced decomposition	Major bloating, skin peeling, organs beyond recognition, bones exposed due to decomposition	2-7 days
Indeterminate	No organ tissues present, skeletal remains, may be mummified	Likely one month or more; if ageing possible it was assumed that the individual died in same year



### **3.4.7 Virus neutralization tests for canine distemper virus**

Jackal and dog sera were tested for VN antibodies to CDV (Chalmers & Baxendale 1994). Briefly, antibody titres were determined by making 4-fold serial serum dilutions starting at 1:8, and incubating them with an equal volume of virus (Bussel strain) suspension at a concentration of between 32 and 316 TCID<sub>50</sub>. After 1hr incubation for neutralization (37°C), the virus/serum mixture was added to freshly seeded VERO cell cultures in 96-well microtitre plates. The plates were incubated for 3-5 days (37°C) and the cell monolayer checked for virus-specific cytopathic effect (CPE) by microscopy. The titre of neutralising antibodies was taken to be the last dilution to inhibit 50% CPE and the cut-off point was determined from the frequency distributions of the titres (log<sub>10</sub>).

### **3.4.8 Statistical analyses**

Logistic regression analyses of CDV seropositivity were performed as described in Chapter 2 (section 2.10), with age class, location, sampling year and sex as explanatory variables. The results of the full models with age class fitted last, including and excluding the age classifications of dogs from Lüderitz in 2003, were compared and the results from the models excluding these dogs presented. The inclusion of the dogs with uncertain age classifications ( $n=19$ ) from Lüderitz did not result in any qualitative changes in the results.

Logistic regression analyses, using sex and age class as explanatory variables, were also used to determine if the numbers of individuals with clinical signs and the numbers of dead individuals varied significantly with age class. Morbidity and mortality were classed as '1' and '0' for the presence or absence of clinical signs or mortality respectively. Age class and any other factors used in these analyses were classed as for the analyses of seropositivity, described in Chapter 2 (section 2.10).

The Wilcoxon rank sum test, a non-parametric test, was used as an alternative to the two sample *t*-test for the analysis of VN titres as the assumptions of normality and constant variance were not met.

### 3.4.9 The basic reproductive number, $R_0$

#### 3.4.9.1 Epidemic curve

An estimate of  $R_0$  for CDV in jackals was obtained from the exponential phase of the epidemic curve (for Cape Cross in 2002) using the equations derived for a closed epidemic of rabies in dogs (Coleman & Dye 1996, Coleman 1999). The epidemic curve could not be constructed for dogs as no detailed data were available on when cases occurred. The incidence during the early stage of the epidemic ( $y_t$ ) is calculated from Equation 1.0

$$y_t \approx ke^{-rt} \quad \text{Equation 1.0}$$

where  $k$  is a constant (the intercept) and  $r$  is the exponential rate of increase in incidence through time. A good approximation of  $R_0$  can be obtained by using  $r$  in Equation 2.0

$$R_0 = 1 + r(lf + g) \quad \text{Equation 2.0}$$

where  $l$  is the latent period of infection,  $f$  is the infective period i.e. the life expectancy of the animal once it is infective or the time to recovery, and  $g$  is the generation time of infection (Equation 3.0) which is the sum of the latent and infective periods.

$$g = l + f \quad \text{Equation 3.0.}$$

### 3.4.9.2 Parameter estimates

#### *Latent period, l*

The latent period is defined as the duration of the period between infection and infectiveness. The spread of virus to the epithelial tissues is associated with the shedding of virus from all body secretions, even in dogs with sub-clinical infections, and this is thought to occur between 8 and 9 days post infection (Greene & Appel 1998); the duration of this period is thought to be fairly constant (Fairchild *et al.*, 1967). The mean latent period and its variance ( $V$ ) were calculated from the study by Appel (1969) in which the latent period lasted between 6 and 9 days. In the absence of further data, the calculations assumed an even distribution of dogs ( $n=64$ ) across the 4 days of the range of the latent period. The mean was used in Equations 2.0 and 3.0 and the variance in Equations 4.0 and 4.1.

#### *Infective period, f*

The infective period was taken as the time to recovery which is associated with the cessation of viral shedding, or death (Greene & Appel 1998). After the latent period, the dogs which mount a good immune response, characterised by a VN antibody titre of over 1:100, have very few or no clinical signs and clear the infection by 14 days post infection (Appel 1969). Dogs with no antibody response are likely to die from severe multisystemic illness by 20 days post infection whilst those with a low antibody response are likely to recover but may shed virus for up to 60 days post infection (Greene & Appel 1998). The range and frequency of the infective period was estimated from a study by Appel (1969) in which 55 dogs were experimentally infected by aerosol. Of these, 27 survived, clearing the infection by 14 days post infection, 26 died between 15-31 days, one died 41 days post infection and one dog died 60 days post infection. In the absence of further data, the calculation of the mean and the variance of the infectious period assumed an even distribution across time of dogs dying between 15 and 31 days post infection. The mean was used in Equations 2.0 and 3.0 and the variance in Equations 4.0 and 4.1.

### *Exponential rate of increase in incidence, r*

An estimate of  $r$  was obtained by fitting Equation 1.0 to the exponential growth in incidence during the CD outbreak in jackals at Cape Cross in 2002. A regression analysis with a Poisson error distribution (SPLUS 2000, MathSoft Inc., (Crawley 1993)) was carried out on the case data for the first 4 months of the outbreak from April to July. Case data consisted of all the mortalities (carcasses found or reported and animals euthanased after capture or at free-range) for which the month of death could be estimated, and individuals with clinical signs captured and released over the 4 month period.

#### *3.4.9.3 95% confidence intervals*

The variance of the  $R_0$  estimate was calculated using standard equations for the calculation of the variance of a function of parameters with known variances (Bulmer 1979). These equations were applied to Equation 2.0 as per the methodology given by Coleman (Coleman 1999) which assumed that there was no covariance between any of the parameters in Equation 2.0.

By substituting the following algebraic abbreviations

$$lf = \alpha,$$

$$r\alpha = \chi,$$

$$g + \chi = \varepsilon \text{ and}$$

$$r\varepsilon = \phi.$$

into Equation 2.0,  $R_0$  is given by  $1 + \phi$ .

From the standard equations for the calculation of the variance applied to Equation 2.0:

$$V(g) = V(l) + V(f)$$

*Equation 4.0*

$$V(\alpha) = \alpha^2 \times \left[ \frac{V(l)}{l^2} + \frac{V(f)}{f^2} \right] \quad \text{Equation 4.1}$$

$$V(\chi) = \chi^2 \times \left[ \frac{V(r)}{r^2} + \frac{V(\alpha)}{\alpha^2} \right] \quad \text{Equation 4.2}$$

$$V(\varepsilon) = V(g) + V(\chi) \quad \text{Equation 4.3}$$

and

$$V(\phi) = \phi^2 \times \left[ \frac{V(r)}{r^2} + \frac{V(\chi)}{\varepsilon^2} \right] \quad \text{Equation 4.4}$$

As  $R_0 = I + \phi$ , so  $V(R_0) = V(\phi)$ .

It is assumed that the SE of  $R_0$  is equivalent to its standard deviation (the square root of  $V$ ) and that this error is normally distributed so that the 95% CI of  $R_0$  is calculated as

$$CI = R_0 \pm (SE \times 1.96) \quad \text{Equation 5.0.}$$

#### 3.4.9.4 Proportion experiencing infection

Estimates of  $R_0$  in jackals and dogs, for Cape Cross and Lüderitz respectively, were obtained from the proportion of individuals experiencing infection in a closed epidemic (Equation 6.0) (Anderson & May 1991, Tompkins *et al.*, 2002). The demographics of the jackal and dog populations were considered negligible as the outbreaks at each location occurred over very small timescales.

$$R_0 \approx -\ln(1 - z) / z \quad \text{Equation 6.0}$$

The proportion which experienced the infection ( $z$ ) was determined from the population size estimates and

- a) the total numbers of cases i.e. mortalities and live individuals with clinical signs, and from
- b) the numbers of seropositive individuals.

In the case of jackals, the proportions of cases and seropositives were only calculated for Cape Cross in 2002 as this was the year of the outbreak and the only location with a population estimate. The proportion which experienced the infection was also calculated using the lower 95% CI value of the population size for comparison as this would result in a more conservative (i.e. larger) value of  $R_0$ .

For dogs, the proportions of cases and seropositives were calculated for Lüderitz in 2003 alone as the sampling of a small number of individuals after the peak of the outbreak in Swakopmund and Walvis Bay would likely result in an inaccurate estimate. In addition, the sequence of the epidemic indicates that Swakopmund and Walvis Bay may not be as isolated as Lüderitz for the introduction of infection by dogs therefore violating the assumption of a closed epidemic.

The upper and lower limits of the 95% CI of the  $R_0$  estimates from the proportions of seropositives and cases were calculated from the upper and lower exact binomial 95% CI values of the proportions,  $z$ , calculated using Epi Info (Dean *et al.*, 1995).

#### 3.4.9.5 Vaccination coverage

Estimates of the percentages of the jackal and dog populations at each location which must be vaccinated in order to prevent an outbreak were estimated using Equation 7.0 (Anderson & May 1991) and each of the  $R_0$  estimates.

$$p_c = \left(1 - \frac{1}{R_0}\right) \times 100 \quad \text{Equation 7.0}$$

The upper and lower 95% CI for estimates of  $p_c$  were calculated from the upper and lower limits of the 95% CI of the  $R_0$  estimates using Equation 7.0.



## **3.5 Results**

### **3.5.1 The temporal and spatial pattern of the CD epidemic in jackals and dogs**

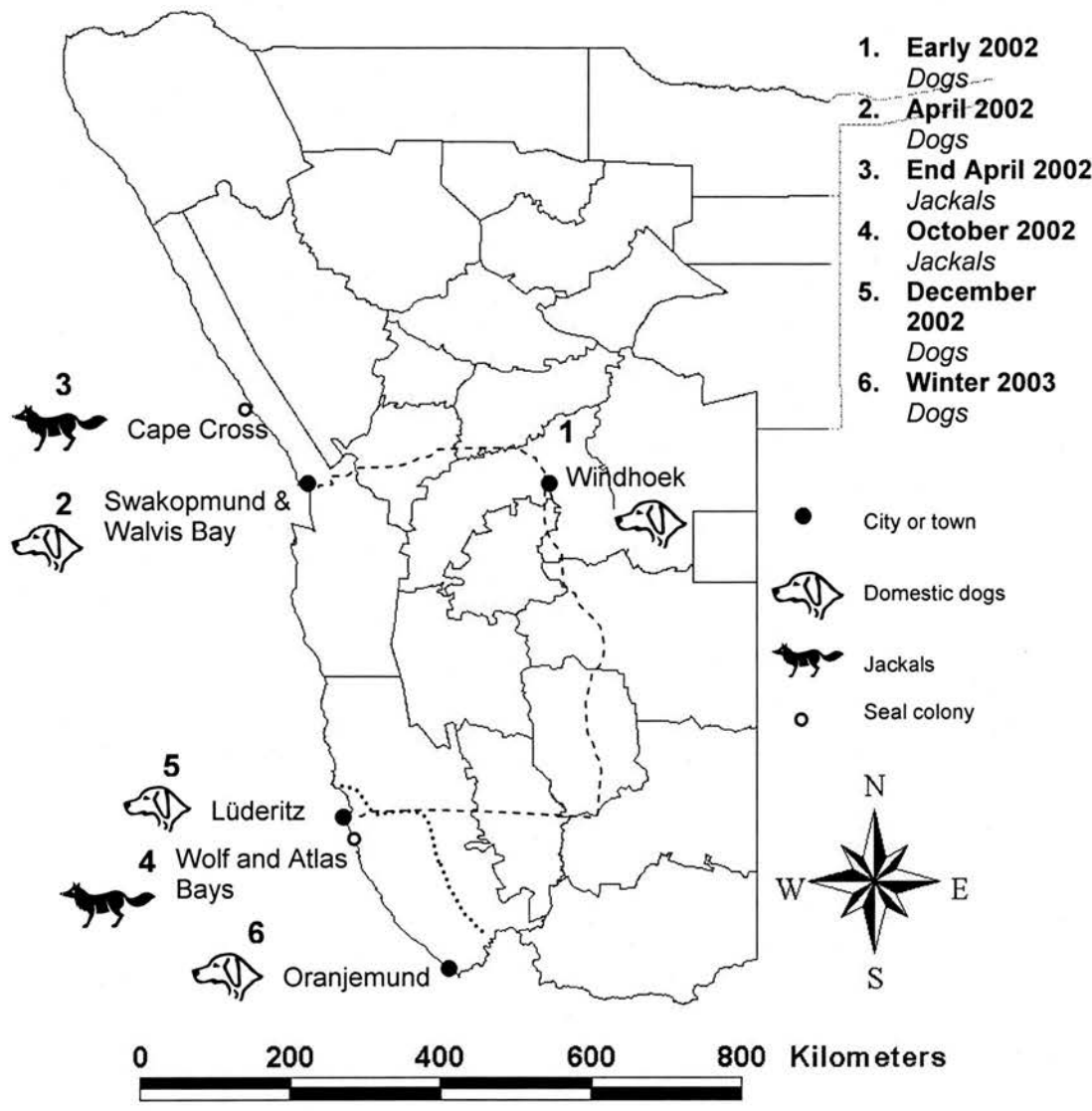
This section presents the temporal and spatial sequence of the CD cases in jackals and dogs as determined from informer reports, cases of morbidity and mortality, confirmed cases, and exposure as determined by serological testing (please see Table 2-14, page 81, for a summary of the sampling by location and year). The results suggest that the CD outbreak followed the spatial and species sequence of transmission as detailed in Figure 3-5.

In 2001, prior to the epidemic, only nine jackal carcasses were recovered, two of which were road kills. None of the jackals ( $n=17$ ) were seropositive and none of these individuals or the domestic dogs ( $n=22$ ) examined before 2002 had any clinical signs consistent with CDV infection, but of the 9 dogs sampled in Walvis Bay in 2001, three were seropositive adults.

In early 2002, an epidemic of CD amongst domestic dogs was reported in the capital city, Windhoek. No vaccinated dogs were reported to have contracted the disease but over 100 non-vaccinated dogs were euthanased and warnings were issued to dog owners (pers. comm. H. Winterbach).

The epidemic then spread to the coast with the occurrence of clinical cases in the dog population of Swakopmund, 260km west of Windhoek. The peak of this epidemic is thought to have occurred between April and May during which a veterinarian reported treating 50 dogs and euthanasing approximately 100, of which only 2-3 were in nearby Walvis Bay (pers. comm. H. Winterbach). Unconfirmed reports estimated that an additional 200 dogs died during the outbreak and that the rubbish dump was "littered with carcasses" (pers. comm. H. Winterbach). Of 18 dogs sampled and examined from Swakopmund in 2002, 11 were seropositive of which three had clinical signs consistent with CDV infection. Of those seropositive, 6 were from the town rubbish dump.

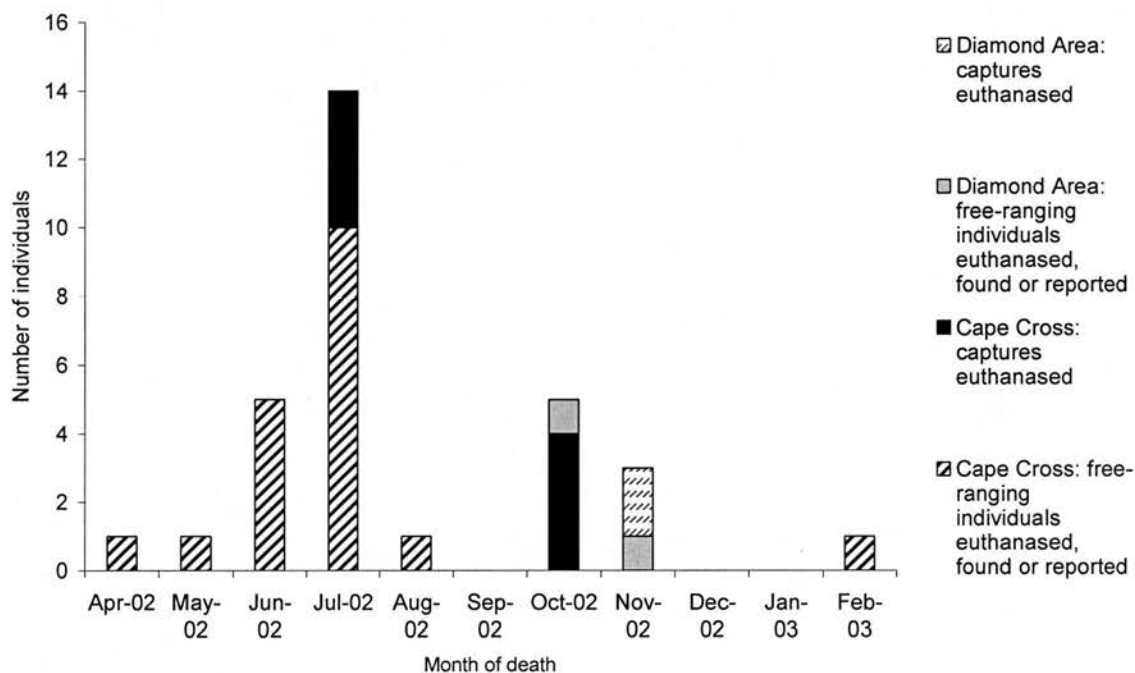
**Figure 3-5: Map of Namibia's magisterial districts showing the sequence of the CD epidemic by species and location, 2002 to 2003.** The highways ( - - ) shown are the major routes linking the capital and the coastal towns. Diamond Area boundary (.....).



In jackals, the index case is likely to have occurred at the end of April 2002, when an individual in very poor condition showing signs of disorientation was euthanased in Walvis Bay (pers. comm. H. Winterbach). At the CCSR, the first potential case of CD in a jackal was an adult female which was shot on the border of the reserve in early May. This animal was described as having no fear of human presence, excessive salivation and other un-specified neurological signs. Neither of these two cases were tested for rabies.

In 2002, the carcasses of 43 free-ranging jackals were reported ( $n=6$ ), recovered ( $n=19$ ) or euthanased ( $n=9$ ) and an additional 9 were euthanased after capture. Seven carcasses were recovered at Cape Cross in early 2003. The month of death was estimated for 31 individuals (Figure 3-6).

**Figure 3-6: The numbers of jackal carcasses for the epidemic period, April 2002 to January 2003.  $n=31$ .**



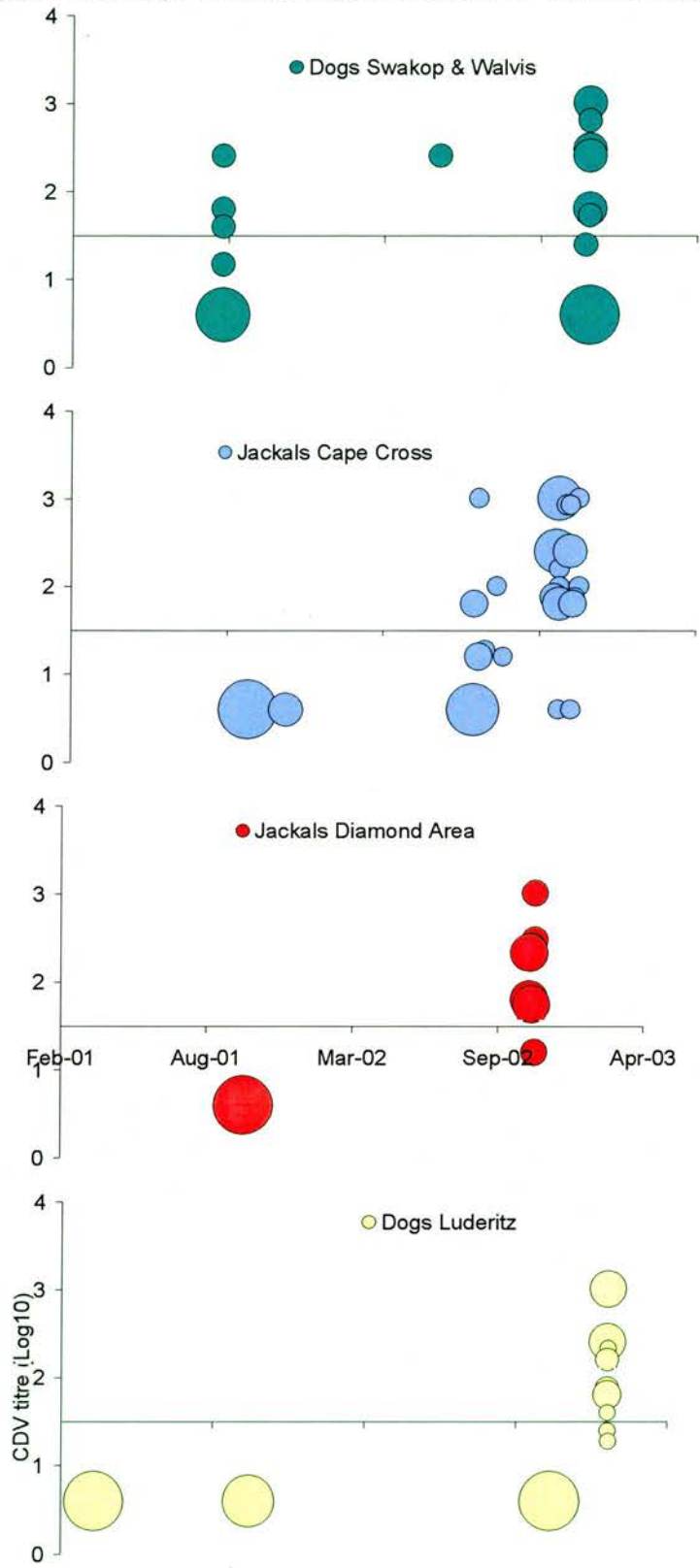
Peak mortality in the Cape Cross population occurred during July 2002 ( $n=14$ ). This was also one of the two months, July and October, in which the greatest number of captured jackals was euthanased. Of the 14 carcasses for which a month of death could not be estimated, 3 came from the Diamond area and 11 from Cape Cross. A year later, in October 2003, no jackals with clinical signs of CDV infection were captured during sampling at Cape Cross but 42% were seropositive ( $n=19$ ). Of two brown hyenas captured during the same period, one was seropositive and did not have any visible clinical signs at the time of capture. A sub-adult male jackal sampled in 2002 was re-captured in 2003 and showed an increase in antibody titre from 1:64 to 1:161.

Jackals with a range of clinical signs consistent with CDV infection, including respiratory problems, seizures and myoclonus, were captured between July and November 2002, at both Cape Cross and the Diamond Area. Of 57 individuals examined for clinical signs in 2002, 46% had one or more clinical signs consistent with CDV infection. At Cape Cross, the numbers of cases with clinical signs followed a pattern similar to that of mortality (Figure 3-6) with the higher proportion of symptomatic individuals in July-August (67%, 12/18) compared to the October-November (38%, 11/29) capture period. Of 10 jackals examined in the Diamond Area in November 2002, 30% had clinical signs.

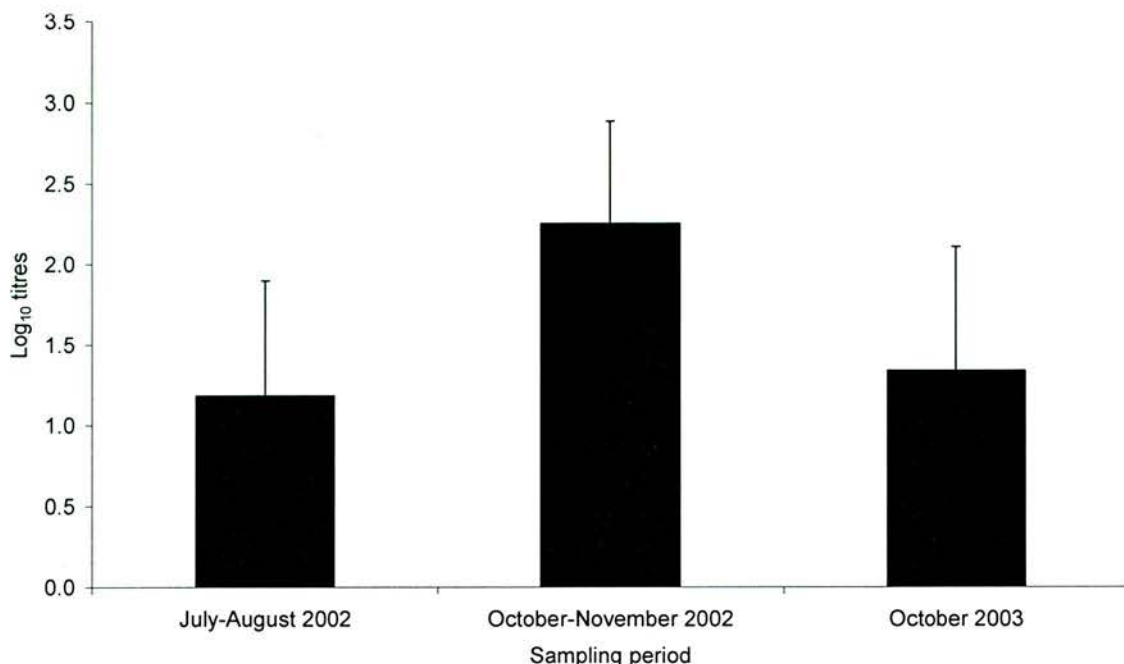
A noticeable decrease in jackal sightings in the second half of 2002 and early 2003 was reported in the Diamond Area at both Baker's Bay (117km south of Lüderitz, 27°47'S/15°31'E) and in the Wolf and Atlas Bay area by staff of the Ministry of Fisheries and research scientists working in the area. Decreased sightings of jackals were also reported at Sandwich Harbour (45km south of Walvis Bay) in January 2003 (pers. comm. R. Simmons).

The course of the epidemic can also be followed by the pattern of the VN titres detected in jackals and dogs sampled over the course of 2001-2003 (Figure 3-7). The titres of VN antibodies detected in jackals sampled at Cape Cross in 2002 reflected the course of the epidemic with a lower average during the period of peak mortality (July-August) but higher levels in October-November of 2002, when the frequency of mortalities decreased (Figure 3-8). Jackals sampled at Cape Cross in October to November 2002 had significantly higher titres compared to those sampled in 2003 (Wilcoxon rank sum test,  $Z=3.75$ ,  $P<0.001$ ) and those sampled in July-August 2002 ( $Z=-3.96$ ,  $P<0.001$ ).

**Figure 3-7: The VN antibody titres to CDV in jackals and dogs, sampled between March 2001 and October 2003. The cut-off point, below which titres are considered negative (log<sub>10</sub> titre of ≥1.5) is indicated by the x axis. n=92 domestic dogs and 90 jackals.**



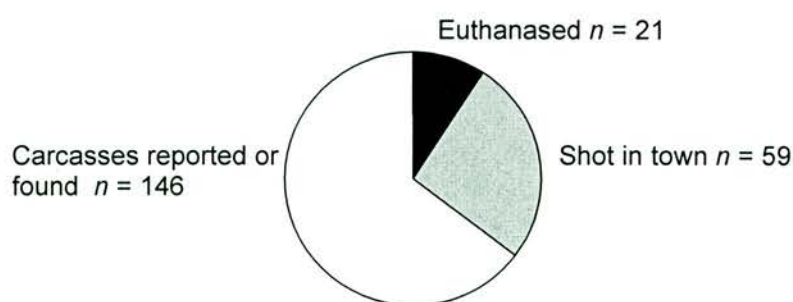
**Figure 3-8: The mean VN antibody titres ( $\log_{10}$ ) for the three main sampling periods at Cape Cross, 2002-2003. Standard deviations are shown above the bars.  $n=64$ .**



Of the 5 dogs sampled in Lüderitz on the 1<sup>st</sup> of October and of the 13 sampled on the 8<sup>th</sup> of November 2002, none were seropositive but one of the latter had myoclonus of the hind legs and was weakened and lethargic. A suspected case of CD was reported in the local SPCA dog kennels on the 24<sup>th</sup> of November (pers. comm. I. Wiesel, research scientist) and two cases of CDV infection were confirmed by histopathology on the 9<sup>th</sup> of January (pers. comm. Dr. F. Mettler, Dr. D. Tinooga). The majority of dog cases in Lüderitz occurred during January 2003, during which two jackals with neurological signs of CDV were sighted at the town rubbish dump. The last reported case was in April (pers. comm. I. Wiesel). Of 22 dogs examined between the 4<sup>th</sup> and the 27<sup>th</sup> of January 2003, 20 had one or more clinical signs consistent with CD infection. A total of 126 carcasses were collected by the Town Council over the same period and an additional estimated 100 dog carcasses were reported but not collected (pers. comm. I. Wiesel, Figure 3-9). Of eight suspected rabies cases during January 2003, three were tested, of which one was confirmed positive. Later in May 2003, reports were received of a similar outbreak in the dogs in Oranjemund, a coastal town 245km south of Lüderitz.



**Figure 3-9: The numbers of domestic dogs shot, euthanased, reported or found dead during the CD epidemic in Lüderitz, 4<sup>th</sup> to 27<sup>th</sup> January 2003.  $n = 226$ .**



For dogs, there was no significant difference between serum antibody titres in those of Swakopmund and Walvis Bay between 2001 and 2002 (Wilcoxon rank sum test,  $Z = -1.71$ ,  $P = 0.0875$ ) and no significant difference ( $Z = -1.79$ ,  $P = 0.074$ ) between dogs of the same towns in 2002 and Lüderitz in 2003.

Officers of the Ministry of Environment and Tourism and veterinarians of the coastal regions questioned about the 2002-3 outbreak had no recollection of a similar outbreak although one informant suggested that a small number of suspected cases of CD may have occurred in the Lüderitz SPCA kennels prior to 2002, but it is not known how long ago this occurred. The state veterinarian responsible for Lüderitz also had no knowledge of any CD outbreak occurring in the area since assuming his position in 1999 (pers. comm. Dr. D. Tinooga).

### **3.5.2 Case descriptions**

Individuals which were diagnosed with CDV infection solely by histopathological examination were selected for case reports. This section presents the clinical signs, post-mortem findings and histopathological findings for 3 jackals and 3 dogs and compares the histopathological changes observed in these two species. The histopathological changes observed in an additional one dog and 7 jackals which were either thought to have died due to CDV infection (were found dead during the epidemic) or were euthanased due to their clinical signs are also considered.



### 3.5.2.1 Jackals

Formalin-fixed tissues from 10 individuals were submitted for testing. The brains from a further 2 jackals, sampled in July 2002, were sent for rabies testing and these were negative; these animals were also seronegative for CDV. In total, histopathological changes consistent with CDV infection were found in three jackals, whereas an additional jackal was CDV positive by immunoperoxidase staining. All the jackals which resulted negative by histopathology and positive by immunoperoxidase were seropositive for CDV.

**Case 1** was a female of less than 1 year of age captured on the 16<sup>th</sup> of July 2002 at Cape Cross. This individual suffered seizures, severe abdominal spasms and could not stand up when approached in the cage. The animal had a condition score of 1 and weighed 5.5kg; there was no mange visible. This individual was seronegative with a titre of 1:16.

The *post mortem* examination revealed signs of hyperventilation in the lung ('crackling' of lung tissue upon compression) and foam in the bronchi. The liver was pale with areas of necrosis. The kidney showed white striations in the medulla and adherence of the capsule.

Liver, lung, kidney, spleen, lymph node, urinary bladder and brain tissues were submitted for examination. The brain showed marked lymphoplasmacytic meningoencephalitis and a few intranuclear eosinophilic inclusions; myelitis with demyelination, astrogliosis, and necrosis were also present. The lung showed marked mucopurulent bronchointerstitial pneumonia with moderate numbers of intracytoplasmic inclusions in the pneumocytes and alveolar macrophages. Some areas of the alveolar walls showed thickening due to congestion and moderate neutrophil infiltration. A number of the epithelial cells of the bladder showed intracytoplasmic eosinophilic inclusions. The lymph node cortex was atrophic with few follicles. Although the white pulp of the spleen was normal and reactive, the red pulp showed moderate erythrophagocytosis and haemosiderosis. The liver was moderately congested with scattered neutrophil infiltration.

**Case 2** was a free-ranging adult female of approximately 6 years of age which was euthanased on the 8<sup>th</sup> of August 2002 at Cape Cross. This animal also had seizures and could not stand up when approached. The animal had a condition score of 1 and was very thin, weighing only 5.4kg; there were no visible mange lesions. This individual was also seronegative with a titre of 1:16.

The *post mortem* examination showed a pale liver with mosaic patterns of necrosis and white striations in the medulla as seen in Case 1.

Lung, liver, kidney, urinary bladder, spleen, lymph node, ovary, uterus, adrenal gland, and brain tissues were submitted for examination. The lung showed interstitial pneumonia with macrophages and inflammatory cells in the alveoli. Round cells and scattered vacuolated cells had accumulated in the liver. The mesenteric lymph node showed haemosiderosis, lymphoid depletion and the presence of multinucleated giant cells. There was also a mild meningoencephalitis in the brain.

**Case 3** was an adult male of over 3 years of age, captured and euthanased on the 19<sup>th</sup> of October at Cape Cross. This individual suffered from seizures, had a condition score of 1 and a body weight of 8.5kg. This individual resulted seropositive with a titre  $\geq 1:1024$ .

The *post mortem* examination revealed a friable liver, adherence of the kidneys' capsules and a large number of cestodes in the small intestine; mange lesions covered approximately 20% of the body.

Numerous tissues were submitted for examination including liver, lymph node, lung, spleen and brain. A tentative diagnosis of CDV infection was made for this individual. There was lymphoid depletion of the mesenteric lymph node, particularly in the cortex. In addition, there were numerous macrophages, and plasma cells and eosinophils. The liver presented multifocal hepatitis and hepatosis with areas of dilated sinuses and cholestasis. There were also isolated foci of inflammatory cells (mainly plasma cells) and foci of hepatocellular necrosis. The Kupffer cells



contained vacuolar masses with unusual staining properties. The spleen showed evidence of extramedullary haematopoiesis. The cerebrum, cerebellum and small intestine did not show any inflammatory or degenerative lesions. The testes showed decreased spermiogenesis.

The remaining 7 jackals which were not diagnosed with CDV infection solely on the basis of the histopathological changes observed showed

- a) very few changes in a small number of tissues, which were not specific to CDV infection ( $n = 6$ ) or,
- b) a small number of changes consistent with CDV infection but in which no inclusion bodies and no changes in the brain were detected ( $n = 1$ ).

But since all these individuals were seropositive (except for the index case from Walvis Bay from which no blood was available) and either died, or were euthanased due to the severity of their signs during the CD outbreak, it is highly likely that CDV infection was the cause of death and the observed clinical signs. Furthermore, inflammation and/or congestion of the small intestine and decreased or arrested spermiogenesis, which are signs consistent with CD infection (Greene & Appel 1998), were seen in three of these cases.

As an example of (a) above, no diagnosis was possible for the index case from Walvis Bay. Liver, kidney, heart and brain tissues were submitted for examination. No lesions were noted in the latter two tissues and the lesions in the liver and kidney (moderate congestion and moderate fatty degeneration) were non-specific.

A free-ranging sub-adult male ('b' above) which showed CNS signs was shot at Cape Cross in July 2002. This jackal showed multifocal interstitial pneumonia, erythrophagocytosis, haemosideriosis and plasma cell proliferation of the mesenteric lymph node, and a mild centrilobular degeneration of the liver. Since the pneumonia was the only specific sign of CDV detected and no lesions were detected in the brain and no inclusion bodies were seen, this individual was not diagnosed with CDV infection on the basis of the histopathological changes.

In addition, viral inclusion bodies were detected in the blood slides of two jackals ( $n=19$ ) sampled in the Diamond Area in November 2002. Both individuals had no visible clinical signs of infection and were in good condition, but only one was seropositive.

#### 3.5.2.2 Domestic dogs

Of the tissues from 4 individuals which were submitted for examination (all from Lüderitz) three had histological changes consistent with CDV infection. Tissues from one of these individuals were also positive by immunoperoxidase staining. Histological findings in the fourth dog included moderate congestion of the brain with meningeal haemorrhages and moderate congestion of the liver and lung tissues with haemorrhages and atelectasis respectively. In this section the histopathological changes observed in the three cases are compared to those observed in the jackals (Table 3-9, page 116). Blood was only available from Case 1 and this resulted seronegative for CDV.

**Case 1** was an adult female which was vaccinated against CDV (Vanguard<sup>®</sup> R Plus 5, Pfizer animal health, USA) shortly before the development of clinical signs. Prior to the 23<sup>rd</sup> of January this animal showed lethargy, loss of appetite, ocular and nasal discharge (on one occasion bloody nasal discharge) but no CNS signs. On the 27<sup>th</sup> the animal was experiencing seizures and myoclonus of the jaw muscles (the characteristic ‘chewing gum fits’); it was euthanased the following day. Another dog on the same premises was euthanased as a suspected rabies case earlier in January, but was not tested.

The animal was in good condition with a score of 5 and no mange was visible. The *post mortem* examination revealed a markedly enlarged spleen, moderately enlarged lymph nodes and possible lung inflammation as indicated by some consolidated areas.

**Case 2** was a three month-old female pup held in the local kennels which developed signs 1-2 days post-vaccination (Vanguard<sup>®</sup> R Plus 5). The animal became lethargic, weak, and unresponsive, with diarrhoea and ocular discharge. The whole carcass was submitted for examination. The *post mortem* showed inflammation of the small intestine and an enlarged spleen. The animal was thin with a condition with a score of 1.

**Case 3** was a 5-6 month old male pup that died on the 23<sup>rd</sup> of January 2003. The whole carcass was submitted for examination; the pup had a condition score of 1. The remaining bitch on the premises showed myoclonus, lethargy, loss of appetite and ocular discharge and was treated by a veterinarian.

The pup's *post mortem* examination revealed pale oral and conjunctival mucosa as well as a severe flea and tick infestation. The lungs showed congestion and white froth in bronchi and trachea. The liver was pale and the bladder contained dark yellow mucoid masses. The cervical lymph nodes and spleen were enlarged.



Table 3-9 provides a comparison of all the histopathological findings from jackal and dog tissues submitted for examination (Dr. F. Mettler, CVL, Windhoek).

**Table 3-9: A comparison of the histopathological changes observed in jackals and dogs.** Jackal ( $n=3$ ) and dog ( $n=3$ ).

Organ	Histopathological changes	Dogs	Jackals
Lung	Mucopurulent interstitial pneumonia	1	1
	Interstitial pneumonia	1	1
	Intracytoplasmic inclusion bodies		1
Spleen	Lymphoid depletion	2	
	with necrosis and haemorrhages	1	
	Haemosideriosis, erythrophagocytosis		1
	Extramedullary haematopoiesis		1
Lymph nodes	Lymphoid depletion	3	3
	with haemosiderosis		1
Brain	Meningoencephalitis		2
	with intranuclear inclusions, myelitis, astrogliosis and necrosis		1
	with demyelination	1	1
	Encephalitis	1	
	Vacuolation cerebellum	1	
Liver	Hepatitis		1
	Hepatositis		1
	Focal necrosis	1	
	Degeneration	1	
	Congestion with neutrophil infiltration		1
	Vacuolated cells		1

### 3.5.3 Immunocytochemistry

Tissues from one dog and one jackal were submitted for staining (Table 3-10). The jackal was one of the 6 individuals which were not diagnosed with CDV by histopathology. This individual was an adult female captured in the Diamond Area in November 2002 which was euthanased because of severe respiratory problems. The dog tissues submitted were from Case 2.

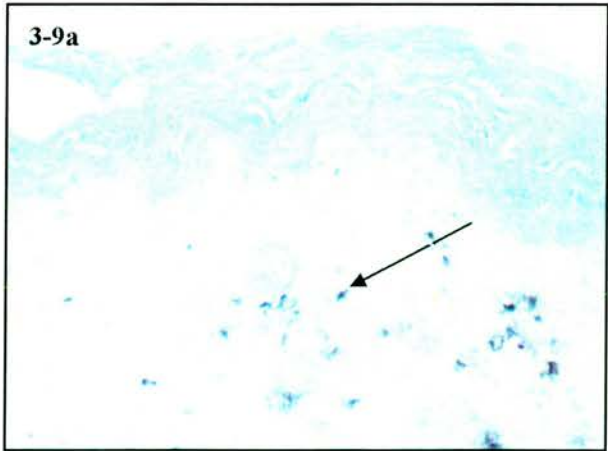
The amount of antigen stained varied in the different tissues. Of the 12 tissues submitted from the jackal, only the lung and mesenteric lymph node were clearly

positive (Figure 3-9a). Of the 14 tissues submitted from the dog, 6 were positive, including the mesenteric lymph node (Figure 3-10a).

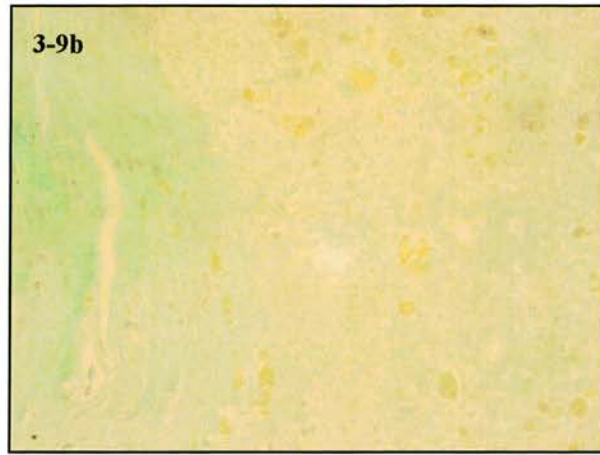
**Table 3-10: The results of the jackal and dog tissues stained by immunoperoxidase technique.** ‘+’ CDV antigen stained, “+” weak positive, - no CD antigen stained, • not sampled.

Tissue	Jackal	Dog
Adrenal gland	-	-
Brachial lymph node	•	-
Cerebellum	•	-
Cerebrum	“+”	-
Heart	-	-
Kidney	-	-
Liver	“+”	+
Lung	+	+
Medulla	•	-
Mesenteric lymph node	+	+
Neck lymph node	•	+
Pre-scapular lymph node	“+”	•
Small intestine	-	+
Spleen	-	+
Thoracic wall	-	•
Urinary bladder	-	-

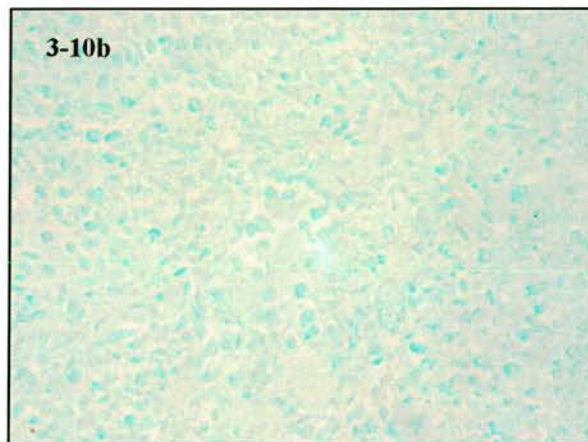
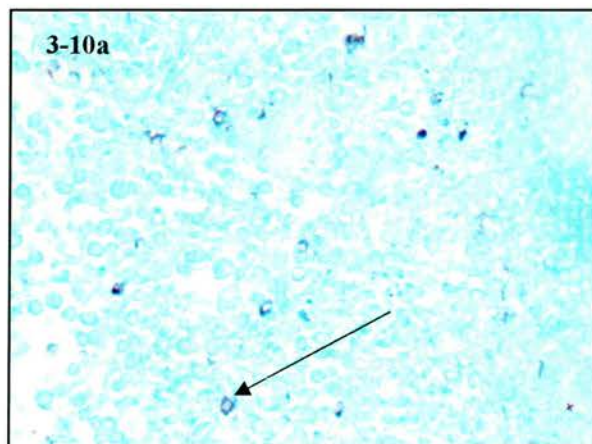
**Figure 3-10: Jackal mesenteric lymph node showing staining of CD antigen.** a) Scattered antigen positive cells present in the cortex of the lymph node, as indicated by the arrow. The location and staining is consistent with cells of lymphoid origin and predominantly cytoplasmic, and b) test control tissue incubated with normal rabbit serum. Original magnification x400.







**Figure 3-11: Dog mesenteric lymph node showing staining of CD antigen.** a) Scattered antigen positive cells present in the cortex of the lymph node, as indicated by the arrow. The location and staining is consistent with cells of lymphoid origin and predominantly cytoplasmic, and b) test control tissue incubated with normal rabbit serum. Original magnification x400.



### 3.5.4 Mortality estimates

**Table 3-11: The maximum and minimum estimated mortality, by location, for jackals and dogs in 2002.**

Location	Species	Minimum (%)	Maximum (%)
CCSR	Jackal	7	16
Swakopmund	Dog	5	8
Lüderitz	Dog	7	11

The jackal population at Cape Cross was estimated at 160 individuals (95% CI 129-205, Chapter 2) after the CD epidemic. The maximum mortality estimate (Table 3-11) can be calculated from the number of individuals euthanased and the carcasses found.

A total of 31 individuals died in 2002 including 26 carcasses for which the month of death was known and 5 carcasses of indeterminate condition for which the age was known. Hence the total population prior to the epidemic was estimated at 191 individuals, resulting in an approximate maximum mortality of 16%. A minimum mortality of 7% can be calculated by assuming those euthanased ( $n=17$ ) would have survived. Extrapolating the mortality estimates to the coastal population of approximately 480 individuals (Chapter 2, section 2.11.1.5), this outbreak may have resulted in the death of at least 77 individuals (assuming that the jackal population estimate is an underestimate of the true population size and applying the upper mortality limit).

The domestic dog population of Swakopmund was estimated at 3654 individuals (section 2.11.2.3). Using the estimate of 300 carcasses (section 3.3.3) the maximum mortality was estimated at 8% and the minimum mortality at 5% by excluding those euthanased (Table 3-11).

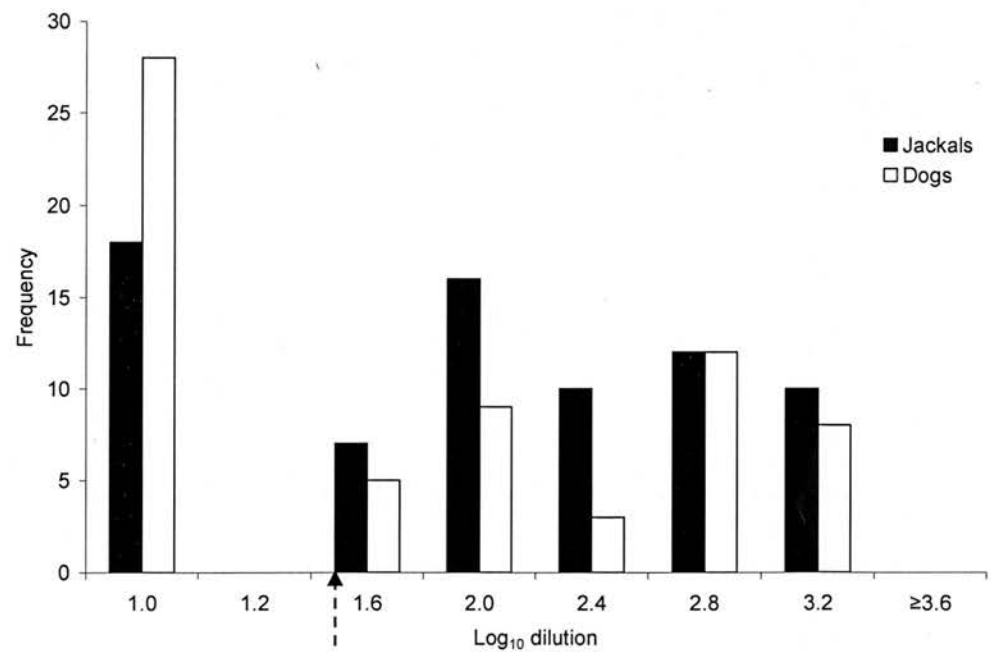
The domestic dog population of Lüderitz was estimated at 2041 individuals (section 2.11.2.3). Minimum and maximum mortality estimates of 7% and 10% can be

obtained from the number reported or found dead ( $n=146$ ) and the total number reported, found and euthanased ( $n=226$ ), respectively (Table 3-11).

### 3.5.5 Serosurvey

A more stringent cut-off point of a reciprocal titre of  $\geq 32$  ( $\log_{10} 1.5$ , indicated by the arrow in Figure 3-12), rather than the standard cut-off of  $>16$  (1.2), was selected for dogs and jackals, according to the titre frequency distributions for individuals sampled in 2002 and 2003 (Figure 3-12). The changes in the  $P$ -values of the logistic regression analyses were not large and the qualitative results unchanged when comparing the same analyses using the two different cut-offs.

**Figure 3-12: The frequency distribution of CDV neutralising antibody titres ( $\log_{10}$ ) in jackals and dogs.** Individuals sampled in 2002 and 2003.  $n=73$  jackals and 65 domestic dogs.



This section presents the results of the logistic regression analysis of exposure to CDV and the age-seroprevalence and age-morbidity and -mortality relationships for jackals and dogs. Summaries of the sampling and results are provided in Tables 3-12 and 3-13 and Figures 3-13 and 3-14.

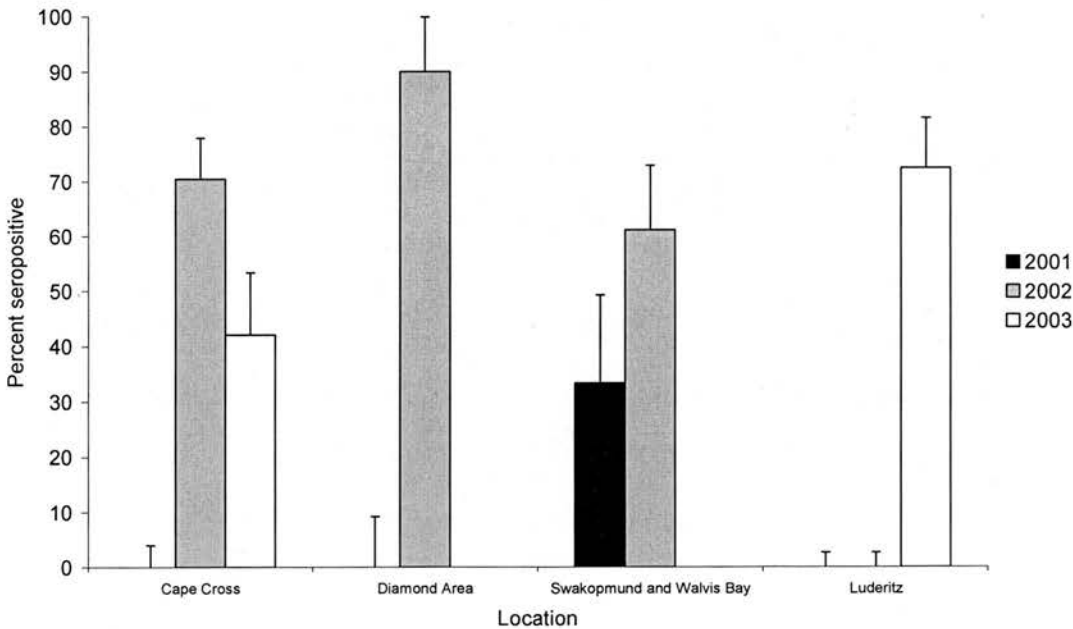
**Table 3-12: The numbers of jackals and dogs tested for exposure to CDV in each age class.** 1 jackal and 14 domestic dogs could not be aged.

Age class (months)	Jackals	Dogs
<12	14	16
12-36	32	11
≥36	43	51
Total	89	78

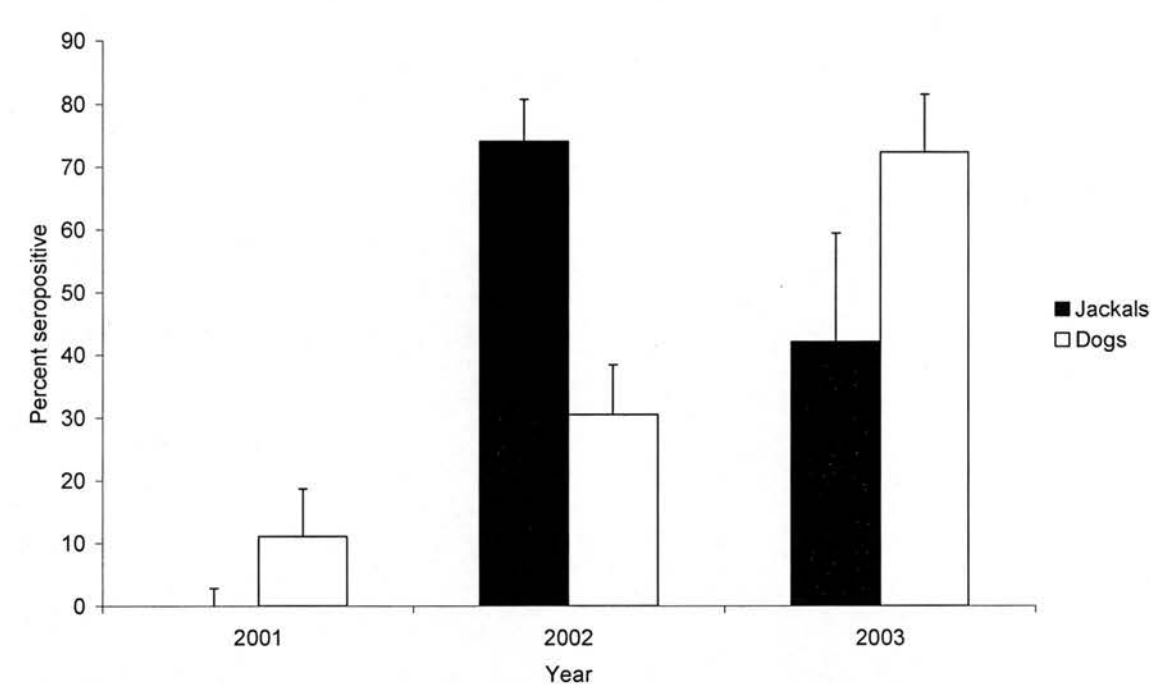
**Table 3-13: The percentages of CDV seropositive jackals and dogs for all locations, 2001, 2002 and 2003. 95% CI in parentheses.**

	2001	2002	2003
Jackals	0.0% (n=17, 0.0-19.5)	74.1% (n=54, 60.3-85.0)	42.1% (n=19, 20.3-66.5)
Dogs	11.1% (n=27, 2.4-29.2)	30.6% (n=36, 16.3-48.1)	72.4% (n=29, 52.8-87.3)

**Figure 3-13: The seroprevalence of CDV in jackals and dogs by location and year.** Standard errors are shown above the bars. n=90 jackals and 92 domestic dogs.



**Figure 3-14: The seroprevalence of CDV in jackals and dogs by year.** Standard errors are shown above the bars.  $n=90$  jackals and 92 domestic dogs.



The results of the logistic regression analysis and age-morbidity and mortality data are presented separately for jackals and dogs. There was no significant difference in the proportion of jackals (53.3%,  $n=90$ , 95% CI 42.5-63.9%) and dogs (38.0%,  $n=92$ , 95% CI 28.1-48.8%) that were seropositive for CDV (full model:  $\chi^2_1=0.527$ ,  $P=0.472$ ).

### 3.5.5.1 Logistic regression analyses, Jackals

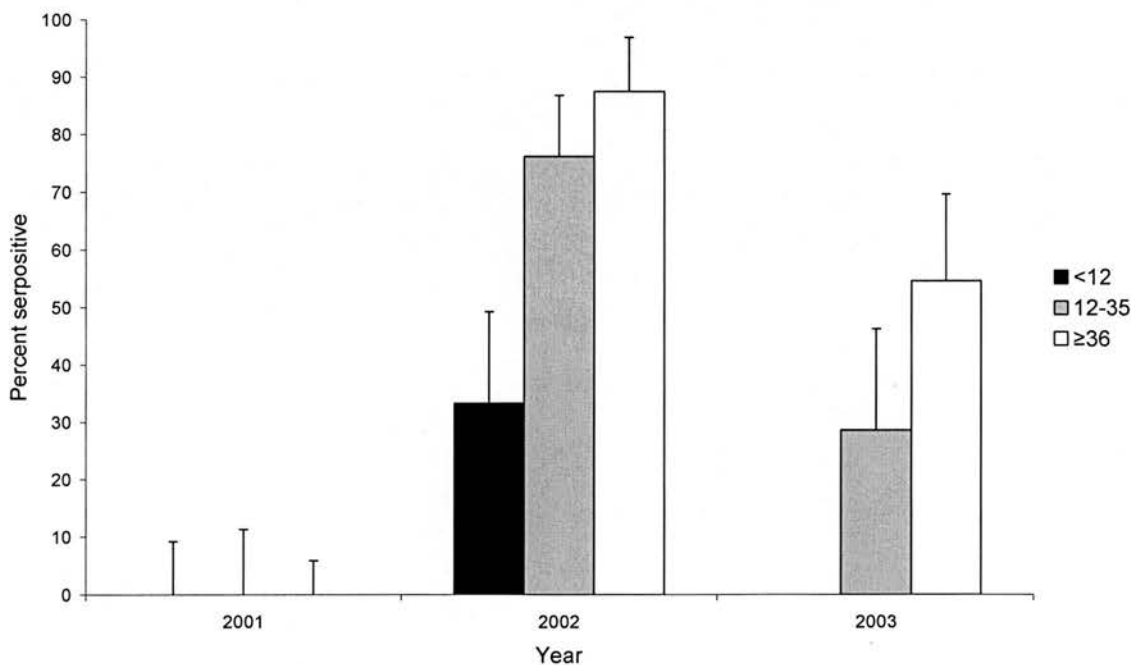
**Table 3-14: Logistic regression models of CDV seropositivity in jackals.**

Term	$\chi^2$	<i>df</i>	Residual <i>df</i>	<i>P</i> -value
Year	39.12	2	84	<0.001
Age	7.87	1	84	0.02
	Coefficient value	s.e.		
Year 2	8.64	13.69		
Year 3	10.55	13.96		
Age class 2	1.81	0.85		
Age class 3	2.71	0.90		

Both age class and year had a significant effect on the proportion of jackals seropositive for CDV (Table 3-14). No jackals were seropositive in 2001 but seroprevalence increased sharply, peaking in 2002 and then decreasing in 2003 (Table 3-13 and Figure 3-14). Taking into account the effect of age, there was a significant effect of year.

After controlling for year, age class was a significant predictor of exposure as the proportion seropositive increased with age class (Figure 3-15). This effect was only significant due to exposure in 2002 ( $\chi^2_2=9.21$ ,  $P=0.010$ ) as no juveniles were captured in 2003 and no animals were seropositive in 2001.

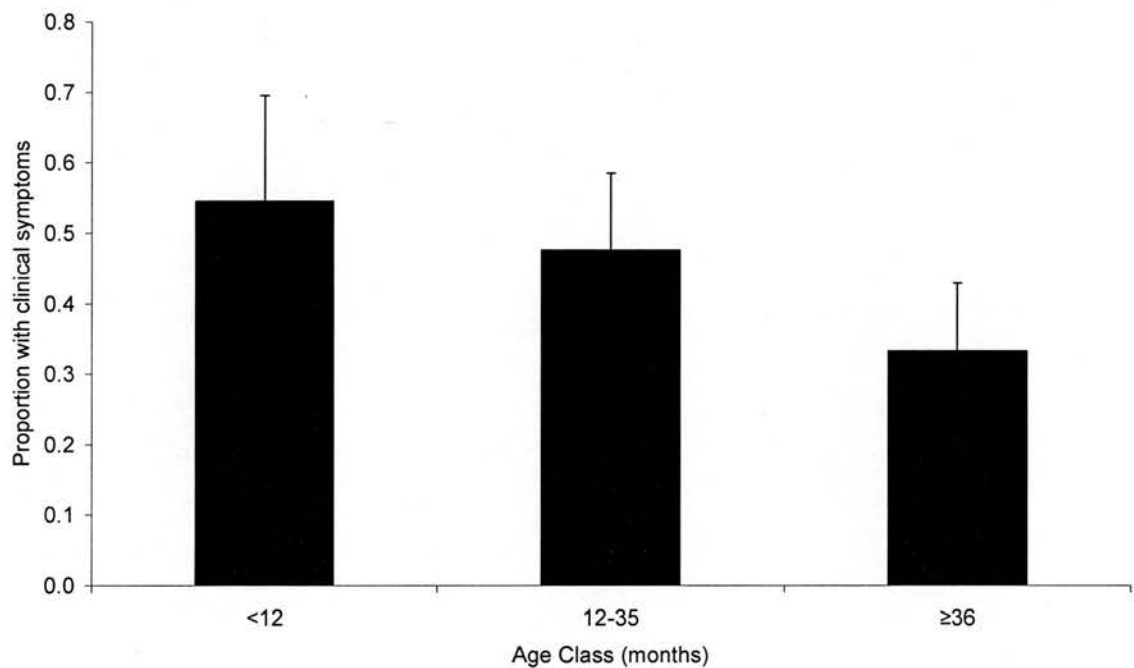
**Figure 3-15: The age seroprevalence of CDV in jackals by year.** Standard errors are shown above the bars. Age classes in months.  $n=89$ .



3.5.5.2 Age-related morbidity, Jackals

There was no significant difference in the proportions of individuals in each age class with clinical signs (Figure 3-16,  $\chi^2_2=3.09$ ,  $P=0.213$ ).

**Figure 3-16: The proportion of jackals in each age class with clinical signs.** Standard errors are shown above the bars.

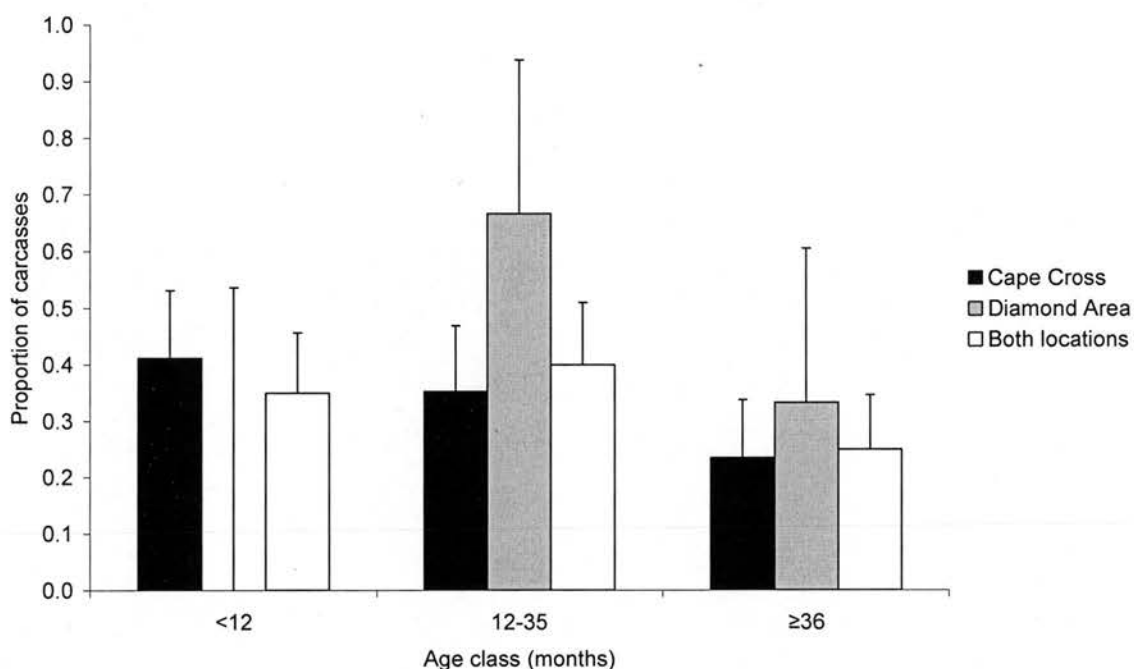


3.5.5.3 Age-related mortality, Jackals

The mortality was reasonably evenly distributed between age classes for both locations combined but at Cape Cross a trend of decreasing mortality with age class is apparent (Figure 3-17). It is not possible to comment on the data from the Diamond Area because of the small sample size; 4 of the 6 carcasses were adults and 2 were sub-adults. Taking into account sex, age class was a significant predictor of mortality ( $\chi^2_2=7.65$ ,  $P=0.022$ ) with fewer deaths in the older age classes.



**Figure 3-17: The proportion of jackal carcasses in each age class by location, 2002.** Proportions are shown for each location separately and for all locations combined. Age classification was possible for 27 of 43 carcasses; excludes the index case from Walvis Bay. Standard errors are shown above the bars.



#### 3.5.5.4 Logistic regression analyses, Domestic dogs

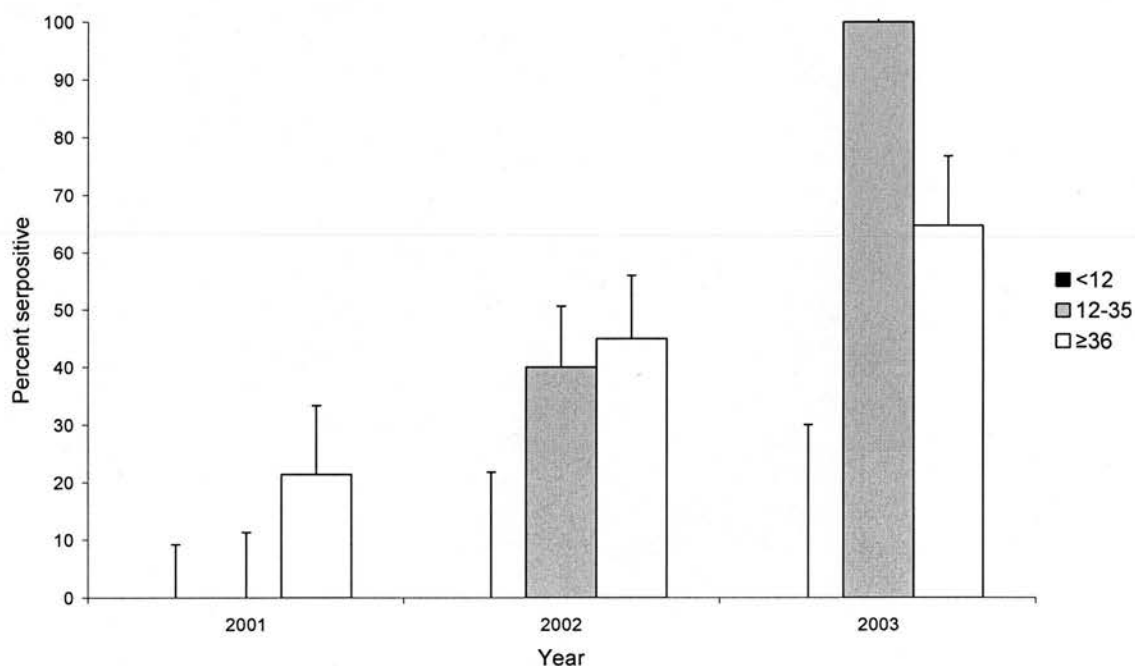
**Table 3-15: Logistic regression models of CDV seropositivity in dogs.**

Term	$\chi^2$	df	Residual df	P-value
Year	34.53	2	72	<0.001
Location	31.85	1	72	<0.001
Age	17.18	2	54	<0.001
	Coefficient value	s.e.		
Year 2	3.08	1.26		
Year 3	13.85	24.61		
Location 2	-12.44	24.59		
Age class 2	11.51	55.89		
Age class 3	11.67	55.87		

Dog age class, year sampled and location all had a significant effect on the proportion seropositive (Table 3-15). After controlling for location and age, year was a significant predictor of exposure. The highest seroprevalence in Swakopmund and

Walvis Bay occurred in 2002 (61.1%) whilst Lüderitz showed the highest prevalence in 2003 (72.4%) (Figure 3-13, page 121). Lüderitz had a significantly lower frequency of seropositives compared to the northern towns (Walvis Bay and Swakopmund combined, Coefficient value -12.44, Table 3-15) and seropositivity increased with age (Figure 3-18).

**Figure 3-18: The age seroprevalence of CDV in dogs by year.** Standard errors are shown above the bars. Age classes in months.



### 3.5.5.5 Age-related morbidity, Domestic dogs

Although the proportions of dogs exhibiting clinical signs increased with age (less than 12 months of age: 0.07, between 12 and 36 months: 0.27 and older than 36 months: 0.29) age class was not a significant predictor of morbidity ( $\chi^2_2=3.61$ ,  $P=0.164$ ). It was not possible to assess age-related mortality in dogs as the great majority of dogs euthanased in Town Council round-ups were not in *extremis* and only one carcass was found dead.

### 3.5.6 The basic reproductive number, $R_0$

#### 3.5.6.1 Epidemic curve

##### *Parameter estimates $l$ and $f$*

The latent period,  $l$ , in dogs was taken to be the mean of 7.5 days post infection and the infective period,  $f$ , was estimated to have a mean of 13.6 days (Table 3-16).

**Table 3-16: A summary of the latent and infective period parameters,  $l$  and  $f$ .** Parameters are in months. Mean values, range, variance, standard error and the numbers of dogs used in the calculations are also given.

Parameter	Mean	Range	Variance ( $V$ )	SE	$n$
$l$	0.25	0.20-0.30	1.25	0.14	64
$f$	0.45	0.27-1.80	0.08	0.04	55

##### *Exponential rate of increase*

The exponential rate of increase in incidence ' $r$ ' was estimated at 0.88 from the regression, the results of which are given in Table 3-17. Equation 1.0 gave a good fit to the exponential phase of the epidemic curve ( $\chi^2_1=12.34$ ,  $P=0.022$ ) (Figure 3-19).

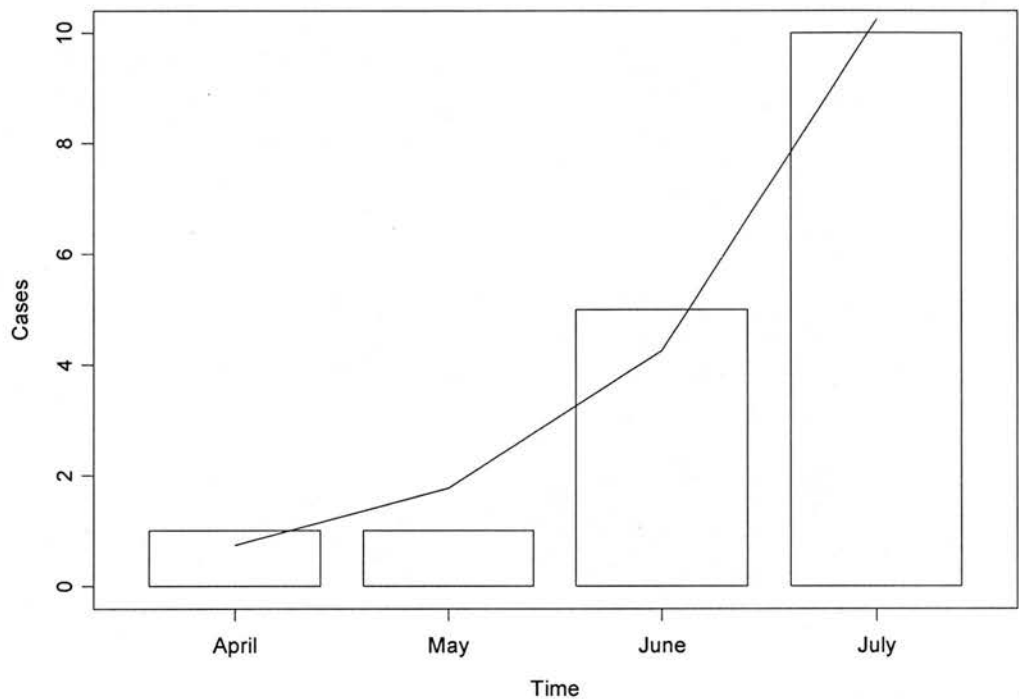
**Table 3-17: The results of the Poisson regression of new CD cases against time in months.**

Term	$df$	Deviance	% Deviance	$P$ -value	Estimated $r$ (SE)
Time	1	12.34	95.29	0.022	0.878 (0.288)
Residual	2	0.61			
Total	3	12.95			

In order to remove any sampling bias, the epidemic curve was constructed solely from cases detected by local veterinarians and MET and hotel staff. The inclusion of cases detected by the project results in 15 cases in July instead of 10 (Figure 3-19) and a slightly higher  $R_0$  estimate of 1.9 ( $V=0.370$ ,  $SE=0.608$ , 95% CI 1.25-2.45).

Equation 1.0 gives a slightly better fit to the plot which includes the project cases with a Deviance of 97.20% and a  $P$ -value of 0.014.

**Figure 3-19: The incidence of CD cases in jackals at Cape Cross, 2002.** The regression line was fitted to the exponential growth period of the epidemic i.e. between the first reported case in Walvis Bay and the month with the highest incidence, July; excluding cases detected by the project.



Using the estimates of  $l$ ,  $f$ , and  $r$  in Equation 2.0, the estimate of  $R_0$  is 1.7 ( $V=0.270$ ,  $SE=0.455$ , 95% CI 0.81-2.59).

### 3.5.6.2 Proportion of cases

A total of 38 jackals experienced mortality ( $n=31$ ) or clinical signs ( $n=7$ ) at Cape Cross in 2002. From the JHE population estimate of 160 individuals the jackal population was estimated at 191 prior to the outbreak, resulting in a proportion ( $z$ ) of 0.199 (95% CI 0.145-0.263) and an estimated  $R_0$  of 1.1 (95% CI 1.08-1.16). The estimate of  $R_0$  from the lower 95% CI value ( $n=129$ ) did not vary significantly as the 95% CI overlapped considerably ( $R_0=1.14$ , 95% CI 1.10-1.20).

A total of 226 dogs were reported dead, found dead or euthanased during the CD outbreak in Lüderitz in January 2003 and an additional 7 dogs were seen with clinical signs bringing the total number of cases to 233. The dog population was estimated 2041 individuals resulting in a proportion ( $z$ ) of 0.114 (95% CI 0.101-0.129).  $R_0$  was calculated to be 1.1 (95% CI= 1.05-1.07).

### 3.5.6.3 Proportion of seropositives

A total of 31 out of 44 jackals sampled for serum from Cape Cross in 2002 were seropositive resulting in a proportion ( $z$ ) of 0.70 (95% CI 0.548-0.832) and an estimate of  $R_0$  of 1.7 (95% CI 1.45-2.15).

A total of 21 out of 29 dogs sampled in Lüderitz in 2003 were seropositive, resulting in a proportion ( $z$ ) of 0.72 (95% CI 0.528-0.873) and an estimate of  $R_0$  of 1.8 (95% CI 1.42-2.36).

A summary of the estimates of  $R_0$  and the respective vaccination coverage levels are given in Tables 3-18 and 3-19. The estimates of  $R_0$  for jackals lie between 1.1 and 1.7 and therefore  $p_c$  lies between 10.3% and 41.2%. The estimates of  $R_0$  for dogs lie between 1.1 and 1.8 and  $p_c$  between 5.7% and 43.8%.

**Table 3-18: Estimates of  $R_0$  for CDV in jackals and dogs by method of calculation, epidemic year and location.**

Species	Year	Location	Method	$R_0$	95% CI
Jackals	2002	CCSR	Cases	1.1	1.08-1.16
	2002	CCSR	Seropositives	1.7	1.45-2.15
	2002	CCSR	Epidemic curve	1.7	0.81-2.59
Dogs	2003	Lüderitz	Cases	1.1	1.05-1.07
	2003	Lüderitz	Seropositives	1.8	1.45-2.15

**Table 3-19: Estimates of  $p_c$  as calculated from the  $R_0$  estimates and the 95% CI for jackals and dogs.** The lower bound of the 95% CI for the epidemic curve is not calculated as the lower 95% CI for  $R_0$  is below 1.0.

Species	Year	Location	Method used to calculate $R_0$	$p_c$	95% CI
Jackals	2002	CCSR	Cases	10.3	7.4-13.8
	2002	CCSR	Seropositives	42.2	31.0-53.4
	2002	CCSR	Epidemic curve	41.2	61.4
Dogs	2003	Lüderitz	Cases	5.66	5.1-6.6
	2003	Lüderitz	Seropositives	43.82	29.6-57.7

### 3.5.7 Results summary tables

**Table 3-20: Summary of CD disease in jackals from the Diamond Area and Cape Cross in 2002.**

Criteria indicating CD infection	Numbers of jackals
Clinical signs of CD	26 of 57
Individuals with one or more of: seizures, myoclonus, respiratory problems and other neurological signs*	
Jackal mortalities	
Carcasses (euthanased, found, reported)	39
Disappearances from observed population of tagged individuals	4 of 51
Estimated mortality	7 to 16%
CDV seroprevalence	74.1%
$n = 54$ samples from captures and euthanased jackals	
Jackals with histopathological lesion(s) consistent with CD infection	4 of 10
Jackals with viral inclusions in tissues (including individual positive by immunoperoxidase staining)	2 of 10
Jackals with viral inclusions in blood, as seen on blood slides	2 of 19
Estimate $R_0$ range	1.1 to 1.7

\* Other neurological signs included depression, disorientation, and inappropriate behavioural responses

Of 57 jackals examined for clinical signs of CDV infection, 4 did not yield blood (2 with clinical signs and 2 without) and none of these 4 were tested by a laboratory test (one or more of serology, histopathology, blood slide examination or

immunohistochemistry). Of the 26 jackals which showed clinical signs of CDV infection, 17 were positive by a lab test (Table 3-21). The relative risk for an individual having clinical signs and being seropositive was 0.82 (95% CI 0.61-1.10,  $\chi^2=1.89$ ,  $P=0.170$ ).

**Table 3-21: The numbers of jackals with or without clinical signs which tested positive or negative for CDV infection.**

Clinical signs	Lab test		<i>Total</i>
	1	0	
1	17	7	24
0	25	4	29
<i>Total</i>	42	11	53

The absence of a correlation between clinical signs and a positive laboratory test could be due to the limited definition of a suspected distemper case (section 2.5.2), the absence of a detectable VN antibody response in acute cases, very mild infections, prior exposure, or a delay in the production of a VN response.



### **3.6 Discussion and conclusions**

#### **3.6.1.1 Canine distemper in jackals and dogs**

Together, several lines of evidence suggest that the disease outbreaks observed in jackals and dogs on the Namibian coast in 2002 and 2003 were caused by CDV. The clinical and histopathological findings in jackals were very similar to those described for dogs in this study and elsewhere (Appel 1987b, Greene & Appel 1998). Furthermore, definitive diagnoses were obtained in both a jackal and a dog by immunocytochemistry.

Natural CDV pathogenesis in dogs has been well characterised and is thought to be similar in wildlife, although the signs of disease may be subtle or not evident at all (Montali *et al.*, 1991). The most common clinical signs in dogs are the neurological signs, followed by cases with both respiratory tract infection and neurological signs; myoclonus is very common in both the limbs and the jaw muscles (Leisewitz *et al.*, 2001). Observations of clinical signs in wild animals during capture and sedation are particularly difficult and although the definition of a suspected CDV case in this study was based on limited observations (section 2.5.2) it was possible to observe some similarities between domestic dogs and jackals. Neurological signs were very common in the jackal population but myoclonus was most frequently seen in the rear quarters and muscles of the abdomen and less frequently in the head. The most common non-neurological symptom in dogs, the mucopurulent oculonasal discharge (Leisewitz *et al.*, 2001), was not seen in the jackals in this study. The other clinical signs observed in jackals, namely abnormal behaviour, ataxia and a lack of fear also occur in foxes and raccoons (Montali *et al.*, 1991).

Dogs that succumb to acute CDV infection between 2-4 weeks post-infection have little or no VN antibody in the serum as the serum antibody titres vary inversely with the severity of the disease (Appel 1987b, Greene & Appel 1998). As in acute cases in dogs, the humoral response was minimal in the jackals (Cases 1 and 2) which showed meningoencephalitis whilst Case 3, in which no brain lesions were detected, had a very high antibody titre. In those cases where a VN antibody response may be

absent other diagnostic tests such as an ELISA which detects actual antigen instead of antibody (Soma *et al.*, 2003) may be more sensitive. The depletion of the lymphatic system and the interstitial pneumonia observed in the jackal cases are also typical of acute CDV infection in dogs (Appel 1987b). Histopathological examinations of the stomach and small intestines were not performed for the majority of the jackals so it is not known if these organs are affected as in domestic dogs. But the occurrence of congestion and inflammation in a number of the post-mortems suggests that the gastrointestinal tract was also affected in this species. The arrested or decreased spermatogenesis observed is likely due to a mild interstitial epididymitis which is commonly seen in dogs (Greene & Appel 1998) and raccoons (Hamir *et al.*, 1992) infected with CDV.

#### 3.6.1.2 Mortality

Mortality caused by CDV is very variable depending on the virus strain, the host species, host age, immune status, and environmental conditions. In dogs mortality, which may reach 50% (Appel 1987b), is highest in susceptible and isolated populations (Greene & Appel 1998). But the mortality in the coastal towns, although likely underestimated in this study, was likely not as high as 50%, which is suggestive of the presence of immunity in the population. The mortality in dogs may have been similar to that seen in CDV outbreaks dogs in Finland (Ek-Kommonen *et al.*, 1997) and Greenland (Bohm *et al.*, 1989) which were both estimated at approximately 30%. The level of mortality in jackals was similar to domestic dogs but likely underestimated as most of the carcasses would not have been reported or recovered.

In dogs, mortality is not thought to be age specific as although dogs under four months of age may be more susceptible, mortality does not differ significantly between juveniles and adults (Krakowka & Koestner 1976). But this does not appear to be the case in the jackals. The observation that mortality is age-specific is supported by the absence of juveniles in captures from 2003 and the presence of healthy seropositive sub-adults and adults in the same year. Jackal pups are born

between June and September with most births occurring in August or September (Ferguson *et al.*, 1983, Apps 2000) hence a high proportion of the 2002 birth cohort would have been alive and likely affected during the outbreak. Coyotes are also thought to show age-specific mortality with pups being more susceptible than juveniles or adults (Williams 2001).

It is highly likely that jackals, like 25-75% of dogs (Greene & Appel 1998), can be asymptomatic and recover from infection, as evidenced by seropositive and asymptomatic individuals in both 2002 and 2003. Dogs with nervous signs usually die, but some recover, sometimes with residual CNS signs, such as, persistent myoclonus (Appel 1987b) and this may explain the presence of jackals with neurological signs at Cape Cross in January and February 2003.

It is thought that CD is unlikely to have a significant effect on coyotes aside from high mortality in the juvenile age class during epidemics (Williams 2001). Jackals in this study may be similar to coyotes in their resilience and ability to recover after an outbreak, as suggested by an increase in the sightings of breeding pairs in 2003 relative to 2002 (pers. comm. S. Funk) and a higher prevalence of exposure relative to the observed mortality. African wild dogs also show resilience to CDV as Tanzanian populations remain stable despite a high prevalence of exposure (Creel *et al.*, 1997).

### 3.6.1.3 *The characteristics of the CDV outbreak*

Several lines of evidence indicate that the CDV outbreak was a large-scale epidemic occurrence involving a naïve jackal population experiencing sporadic outbreaks and not an endemic state.

First, analysis of the serological data indicates that seroprevalence patterns in jackals were very variable over the years, with no seropositive jackals detected in 2001. And second, although a low seroprevalence could have gone undetected in this population due to the small sample size in 2001 (at Cape Cross, in a population of 191 jackals,

41 positive individuals could have gone undetected) there were no jackals with clinical signs of CDV infection in 2001, or 2003. Third, the high mortality experienced by the jackal population in this study is typical of disease which is periodically re-introduced into susceptible and isolated populations of dogs or wildlife (Alexander & Appel 1994, Greene & Appel 1998). Fourth, the absence of a trend in age for the occurrence of clinical signs in the jackals is indicative of a naïve population in which all ages were affected. Lastly, as high titres are evidence of either repeated exposure or a recent infection, if CDV was persisting in the jackal population one would expect titres in 2003 to be similar to those in 2002, but they are significantly lower.

The pattern of increasing seroprevalence with age in the jackal population could be explained by a) a constant force of infection in an endemic area, b) differential rates of exposure in a population experiencing sporadic outbreaks or c), an increase in disease resistance with age. The variation in seroprevalence between years and the high mortality suggest that this is an epidemic occurrence. There may be differential rates of exposure in the jackal population, and this is investigated in Chapter 6, but there is no evidence to suggest that sporadic outbreaks of CD occur in the coastal jackal population. Hence the increase in seroprevalence with age is likely due to the age specific mortality discussed above.

In dogs, the reasons for the increase in seroprevalence with age are unclear. It is not likely due to a constant force of infection in an endemic persistence as the increase in seroprevalence is clearly due to an epidemic. The small sample size in 2001 cannot distinguish between exposure to CDV circulating in the population at the time of sampling and seropositivity due to survival from a previous outbreak. Variation in the rates of exposure in the different coastal towns, possibly due to differences in dog population size, and the bias introduced from sampling after the epidemic may have resulted in this trend.

#### 3.6.1.4 *Transmission routes*

The CD outbreak is thought to have started in Windhoek in early 2002 (Figure 3-5, page 105), and then spread to the domestic dogs of the towns of Swakopmund and Walvis Bay, the incidence peaking in these localities during March-April 2002. The first un-confirmed case in jackals occurred at the end of April in Walvis Bay and CD spread very rapidly through the jackal population of the CCSR during the winter months, starting with the first suspected case in May 2002. By November 2002 the outbreak had already spread through the jackal population of the Diamond Area but domestic dog cases were only reported at the end of December with the majority of cases occurring in January 2003. It is thought that CDV then spread to the domestic dogs of Oranjemund in the winter of 2003. This pattern of spread, from foci of large domestic dog populations to wildlife is similar to the pattern of spread of the 1990 CDV epidemic in Kenya which is thought to have originated in the dogs of the capital Nairobi, spread to the domestic dogs of the Masai-Mara region, and from there spilled-over into wildlife (Alexander & Appel 1994).

The spread CDV from Windhoek to Swakopmund and Walvis Bay was likely due to the transportation of infected animal(s) along the highly trafficked route linking Swakopmund, Usakos, and Okahandja. But the spread north into the jackal population of Cape Cross was probably due to the movement of infected jackals which may have contracted CDV from dogs in the vicinity of Swakopmund or Walvis Bay. No dogs are allowed in the reserve and none were shot there in 2002, before the outbreak (pers. comm. V. Kotze, Park Warden). The occurrence of the index case in Walvis Bay and the shooting of jackals at the Swakopmund cemetery between March and May 2002 (pers. comm. H. Winterbach) indicate that jackals do enter the towns. The rubbish dumps, at which seropositive dogs were also found (Swakopmund), are other potential points for inter-species transmission, to which jackals are likely attracted when food availability at the colony is low i.e. September and October. Dogs have been known to enter the CCSR but this is not thought to be a frequent occurrence (pers. comm. V. Kotze, Ministry of Environment). There are also numerous campsites and fishing points between Cape Cross and Swakopmund at which jackals and dogs may meet – as demonstrated by the sighting of a radio-

collared jackal foraging at Myl 72, 60km north of Swakopmund, and a report of a jackal attacking a domestic dog at Myl 18 in January 2003.

The movements of domestic dogs are likely more restricted to the vicinity of the coastal towns, due to their dependence on humans, than those of jackals, which are capable of dispersing and foraging over large distances (Ferguson *et al.*, 1988). Extensive movements (>200km) of tagged jackals north and south along the coast have been recorded (van Dreyer & Nel 1990). It is therefore likely that CD epidemic spread south into the Diamond Area by contact between jackals.

Several lines of evidence support this. Firstly, if the outbreak in Lüderitz had been caused by the transportation of infected dog(s) from another town, one would have expected the outbreak to occur much earlier and not one year after the outbreak in Windhoek. Secondly, a decrease in the number of jackal sightings at Sandwich Harbour in early 2003 is consistent with increased mortality in this area. Thirdly, the capture of infected and symptomatic jackals and the discovery of an unusually high number of carcasses in the Diamond Area at the start of November 2002, indicate that a CD epidemic was underway well before the first suspected case in dogs in Lüderitz. Furthermore, all the dogs sampled in Lüderitz in October and November were seronegative. In this area, the smaller distance between Lüderitz and the colony made the spill-over into dogs relatively easy. Domestic dogs are known to enter the area on a regular basis (pers. comms. T. Cooper, Ministry of Environment, S. Hugo, NamDeb), making contact with both jackals and brown hyenas which in turn, have been sighted at the rubbish dump, the seal factory and in the township area (pers. comm. I. Wiesel).

Finally, the rapid spread of CDV through the jackal population was likely assisted not only by the high population density, but also by the introduction of the virus during the mating season, June-August (Apps 2000) which is when contact rates are likely to increase due to the defence of territory and mating activities. The mating season is thought to lead to an increase in the occurrence of rabies in jackals (Loveridge & Macdonald 2001) and is associated with peak prevalences of CDV in



raccoons in the USA and stone martens (*Martes foina*) in Europe (Williams 2001). Nutrition in dogs is thought to play a role in the outcome of infection (Appel 1970) and the decline in jackal condition associated with winter months prior to the birth of the seal pups in November may have contributed to increase mortality.

It is unclear why, despite the high population density and the ease of transmission of CDV, that the seroprevalence in the jackal population in 2002 was not closer to 100%. Horizontal transmission of CDV is likely modulated by behavioural interactions and the social structure of the population and these factors may form the basis of the perceived natural 'resilience' of wild populations to infections such as rabies (McKenzie 1993, Loveridge & Macdonald 2001).

#### 3.6.1.5 The basic reproductive number, $R_0$

This study provides the first point estimates of  $R_0$  for CDV in terrestrial carnivores which ranged between 1.1 and 1.7 in jackals and between 1.1 and 1.8 in dogs. The estimates of  $R_0$  fall within the 95% confidence limits of  $R_0$  estimates derived from rabies epidemics, also caused by a highly contagious RNA virus (Coleman & Dye 1996). The estimates obtained from the proportion seropositive and the epidemic curve in jackals and dogs differ in that the lower 95% CI for the latter estimate falls below 1.0. The inclusion of the cases detected by the project over the same period results in a slightly higher estimate of  $R_0$  whose confidence intervals are above 1.0. For the purposes of vaccination it is best to slightly overestimate  $R_0$  as to not under-vaccinate and therefore the higher estimate of  $R_0$  is considered more valid.

The model developed by Coleman (1996) and used here to estimate  $R_0$  from the epidemic curve, treats the epidemic as a discrete event occurring over a timescale at which the demographic processes are not significant. The demographic processes play an important role in determining the persistence of a pathogen particularly through the regulation of the input of new susceptibles (Dye *et al.*, 1995, Heesterbeek & Roberts 1995). In this case, the exponential growth of the CDV epidemic is thought to have lasted 16 weeks, which occurred before the whelping of



the jackals in this area and the introduction of new susceptibles from birth. It therefore seems reasonable, assuming negligible emigration and immigration over this period, to consider the population as closed for this estimation of  $R_0$ .

The calculations from the proportions that experienced infection also assume that the epidemic has run its course and done so quickly compared to the time-scale of the host demographic change. But these estimates include the whelping period of the jackals, as all the cases and seropositives from the 2002 study period were included in the calculations. In the case of the  $R_0$  estimate for dogs, the data used span only one month which is a relatively short time period but this is not an accurate measure of the final proportions affected as cases also occurred between February and April which could not be recorded.

The values of  $R_0$  derived from the fraction affected or infected are influenced by biases affecting both the nominator and denominator of the fraction  $z$ . The estimates derived from the numbers of cases are likely to be particularly unreliable as they are dependent on the discovery of the jackal carcasses and the identification of animals with clinical signs as suspected cases of CD which will result in the underestimation of  $R_0$ . The JHE jackal population estimate (sections 2.5.3.2 and 2.11.1.5) is dependent upon the numbers ear-tagged, the numbers of surveys and the assumptions behind the JHE calculations (Neal *et al.*, 1993). The cage traps were less efficient than the footholds (section 2.11.1.2) and therefore decreased the fraction of the population ear-tagged. Violation of the assumption of independent sighting probability by the formation of groups of jackals can bias the population estimate and increase the coverage of the 95% CI. Visibility biases and result in an underestimation of population size and therefore an overestimate of  $R_0$ . Hence it seems reasonable to consider the epidemic curve estimate of  $R_0$  in jackals to be the more reliable figure.

Although there was no way of confirming this at the time of writing, it is possible that the domestic dog population estimates are overestimates as illustrated by the difference between the guestimate provided by I. Weisel for Lüderitz and the

estimate derived from the human:dog ratio. This would result in an overestimate of  $R_0$  but in the absence of population estimates for the coastal towns it is not possible to obtain more accurate estimates of  $R_0$ . For the purposes of vaccination, a moderately overestimated  $R_0$  is not necessarily a serious problem as it is better to err conservatively. But if  $R_0$  is indeed smaller than estimated in this study it could mean that CDV is less likely to spillover between jackals and dogs and the probability of persistence in either species will be reduced.

The data used to reconstruct the epidemic curve includes unconfirmed deaths and one case which was presumed dead after sitings ceased. But the occurrence of clinical signs consistent with CDV infection and the death of these individuals during the CD outbreak strongly suggest infection with CDV. The use of data from domestic dogs for the estimation of the latent and infectious periods does assume that the pathology of the disease is similar in dogs and jackals but this is a reasonable assumption in light of their close phylogenetic relationship (Xiaoming *et al.*, 2004). Assuming a high sensitivity and specificity of the fluorescent antibody technique used by Appel (1969), the estimates of the latent and infectious periods are subject to a degree of error for a number of other reasons. Firstly, data from individual dogs was not available from the study by Appel (1969) and therefore assumptions had to be made regarding the distribution of individuals across these periods. Secondly, the dogs used in this study (Appel 1969) were infected via aerosol with  $\pm 100$  median infective doses ( $ID_{50}$ ) which is likely higher than the infective dosages received under natural conditions. The infective dose will likely affect the duration of the latent and infective periods. The longer the latent and infectious periods the larger the  $R_0$  but there is no comprehensive dataset for natural infections in dogs with which one can estimate these parameters. By attaching a range and CI to the estimates of  $p_c$  and  $R_0$  an attempt has been made to include the uncertainties associated with each of the parameters.

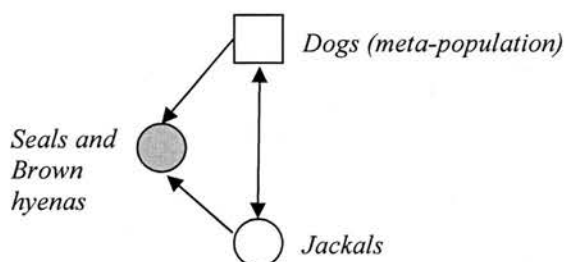
The eradication of CDV in jackals would be warranted if the risk of significant mortality in the seals were high. This would require the vaccination of, conservatively, 50% of the coastal jackal population. This may be possible in the

Cape Cross area by intensive darting and capture, as the jackals are not persecuted and cluster around one area. But vaccination coverage of this level poses further problems elsewhere on the coast where jackals exist at a lower density. But before vaccination is undertaken, studies will have to ascertain a) whether control in the jackal population is warranted and b) the safety, efficacy and duration of vaccine-induced immunity in jackals. Similarly, to prevent an outbreak in dogs in Lüderitz, 44% of the population must be vaccinated. Vaccination in resource poor communities in Namibia is likely to be below the level necessary to prevent another outbreak. The vaccination of domestic dogs against CDV in Lüderitz in 2003 cost N\$50 (approximately £5) per animal. Vaccination coverage against rabies, a disease of much greater concern, for which vaccination is provided free of charge, is only 12% (Sorin & Mvula 2001).

### 3.6.1.6 *The roles played by jackals and dogs*

As the first description of an epidemic of CDV in free-ranging jackals, this study confirms the suspicion raised by many serosurveys, that the jackal can act as an effective transmitter of CDV to both domestic dogs and wildlife (Figure 3-20). But the jackal population in this study system is likely not a maintenance population for CDV, as indicated by several lines of evidence.

**Figure 3-20: The jackal population as a source, and not maintenance population, for CDV.**



First, the total coastal jackal population is well below the CCS necessary to maintain a morbillivirus (Black 1966). Second, the turnover of susceptibles in this species, which only breeds once a year and suffers high pup mortality (Apps 2000), is very

low compared to that of domestic dog populations which breed all year round. Furthermore, it is possible that like dogs, jackals which recover from infection are immune for life. These factors resulted in the CD epidemic 'burning' rapidly through the population, north and south along the coast, and likely fading out due to the lack of susceptible individuals.

The role played by the coastal dog population, however, is unclear. One possibility is that the populations of the coast and towns further inland constitute a maintenance community. The populations of the coastal towns and the coastal population as a whole are both below the CCS for the maintenance of a morbillivirus between epidemics; the CCS for measles in humans is estimated to be at least 300,000 individuals (Black 1966). A meta-population could promote the persistence of a morbillivirus, such as CDV, by allowing epidemics to occur asynchronously in the different sub-populations or towns (Bolker & Grenfell 1996). Distemper may 'fade-out' (Anderson & May 1991) after epidemics in the sub-populations but, as long as these are linked, via for example the transportation of infected dogs, CDV will be maintained in the meta-population as a whole; the dog population of Namibia is likely larger than the required CCS (please see Table 2-13, page 80). The introduction of CDV from South Africa and other neighbouring countries may also contribute to the persistence of CD in Namibia. South Africa has a much larger dog population, conservatively estimated at 4 million, the majority of which is not vaccinated against CDV (Leisewitz *et al.*, 2001). An alternative possibility is that CDV *does* persist in those (coastal) dog populations which are below the CCS. There is some debate about whether models of microparasite persistence result in unrealistically large CCSs and persistence is not well understood (Dye *et al.*, 1995).

The existence of a maintenance community, be it outside of the coastal system, is of great concern. The coastal system can be likened to a protected area or national park, bound on one side by the ocean and on the other by the Namib Desert. The 'park' is made vulnerable to disease by the few points through which CDV can be introduced i.e. the towns of Swakopmund, Walvis Bay and Lüderitz. This introduction may be infrequent but once in the area, CDV spreads very rapidly and results in high

mortality. Species other than jackals and dogs may be a risk from CDV and 'spill-back' into domestic animal populations is of great concern. Once in the coastal system jackals can facilitate the rapid spread of CDV north and south along the coast after which the infection may spill-over to inland populations. A brown hyena from Cape Cross, a species only recently removed from the 'endangered' status on the IUCN list, was seropositive indicating that this species can be exposed to CDV. But the effect of CDV in this population has not been studied; brown hyenas may also suffer significant mortality from CDV as this has been documented for spotted hyenas (*Crocuta crocuta*) elsewhere (Haas *et al.*, 1996). The potential effect on the seal population, which is of significant economic value due to the harvests and tourism, is also of great concern and this is discussed further in Chapter 4.

Namibia's economy is almost entirely reliant on natural resources and tourism. The national parks, game reserves and other protected areas are central to the rapidly growing tourism industry (Brown 1996, Schoeman 1996). The country currently harbours healthy populations of endangered species such as the cheetah and the wild dog, and preventive measures, such as the vaccination of domestic dogs, are essential in order to avert a CD epidemic with potentially serious consequences, such as that which occurred in the Serengeti. Further research is required in areas such as the Etosha National Park, to effectively identify the potential sources of CDV and to target control measures.

As seen in the Serengeti, the dynamics of CDV are constantly changing, and wildlife have come to play a far more central role in the transmission and maintenance of CDV since its spillover from domestic dogs. This study has provided merely a 'snapshot' of the dynamics in the coastal system and cannot predict what changes may occur. A change in virulence or the occurrence of cofactors such as pollution or starvation which can lower the herd immunity, may result in significant mortality for the seal population and other species. But it is likely that another epidemic of CD will occur in the jackal and dog populations once the numbers of susceptibles have recovered; unpublished data indicates that CD epidemics in dogs of cities elsewhere in Africa occur once every 5 years (Alexander & Appel 1994).

**Chapter 4:  
Morbilliviruses and  
the Namibian seal  
population**

## 4.1 Abstract

Morbilliviruses are widely distributed in some marine mammal populations and CDV and PDV infections have resulted in severe mass mortalities in pinniped populations. Based on a previous serological study, an unidentified morbillivirus is thought to be endemic in the Cape fur seal population of Namibia, possibly acting as a co-factor, together with *Klebsiella* spp. infections, to cause low levels of mortality. In 2002, during the present study, this seal population was at risk from the spill-over of CDV from an epidemic in dogs and jackals of the Namibian coast. A longitudinal (2001-2002) and extensive cross-sectional serosurvey was conducted to test for antibodies to CDV, PDV, DMV, CHV and CAV-1 in pup and adult seals (ranging in age from 8 months year to >14 years), from the two largest colonies in Namibia. The very low prevalence of exposure to CDV (0.2%,  $n=570$ , 95% CI 0.0-1.0) and the absence of exposure to PDV and DMV (0.0%,  $n=167$ , 95% CI 0.0-2.2) indicates that a morbillivirus is not likely to be endemic in this population and that the CD epidemic in jackals and dogs did not result in extensive exposure in the seal population in 2002. However, a herpesvirus and an adenovirus may be circulating in the seal population as the prevalence of exposure was 11.4% ( $n=290$ , 95% CI 8.0-15.6) and 22.4% ( $n=80$ , 95% CI 13.9-33.2) respectively. It appears, based on these results, that a morbillivirus is not a factor in the yearly mortalities in this seal population. The possible reasons for the low exposure and the risk posed by morbilliviruses to this population are discussed.



## 4.2 Introduction

Since the outbreak of PDV in the harbour and grey seals of the North Sea in 1988 (Rima *et al.*, 1992) numerous studies, some of them retrospective (for example Ross *et al.* 1992), have demonstrated widespread exposure to a number of morbilliviruses in aquatic species, namely PDV, CDV, DMV and PMV (Table 4-1).

**Table 4-1: Examples of the exposure to, and impact of, morbilliviral infections in aquatic species.** 'Exposure' – detection of antibodies, 'Infection' – detection of antigen; in some cases the effect on the population was not determined. MV: unidentified morbillivirus.

Species	Locality	Virus, evidence for exposure, estimated mortality in epidemic	Reference
Striped dolphins ( <i>Stenella coeruleoalba</i> )	Various countries, Mediterranean	DMV Infection, Population crash ≥600	(Domingo <i>et al.</i> , 1990)
Fin whales ( <i>Balaenoptera physalus</i> )	Belgium & France	MV Infection	(Jauniaux <i>et al.</i> , 2000)
Crab-eater seals ( <i>Lobodon carcinophagus</i> )	Antarctica	CDV Population crash ≥6000	(Bengston <i>et al.</i> , 1991, Barrett 1999, Kennedy <i>et al.</i> , 2000)
Harbour seals ( <i>Phoca vitulina</i> )	North Sea	PDV Population crash ≥17,000	(Osterhaus 1988, Dietz <i>et al.</i> , 1989b, Heide-Jorgenson & Harkonen 1992)
Grey seal ( <i>Halichoerus grypus</i> )	Canada	Exposure	(Henderson <i>et al.</i> , 1992, Ross <i>et al.</i> , 1992)
Ringed seal ( <i>Phoca hispida</i> )			
Harp seal ( <i>Pagophilus groenlandia</i> )			
Hooded seal ( <i>Cystophora cristata</i> )			
Mediterranean monk seals ( <i>Monachus monachus</i> )	Mauritania	DMV (toxins) Population crash ≥150	(Hernandez <i>et al.</i> , 1998, Osterhaus <i>et al.</i> , 1998, van de Bildt <i>et al.</i> , 1999)

**Table 4-1 continued: Examples of the exposure to, and impact of, morbilliviral infections in aquatic species.**

Species	Locality	Virus, evidence for exposure, estimated mortality in epidemic	Reference
Harbour porpoise ( <i>Phocoena phocoena</i> )	Northwest Europe	DMV Infection, Population crash ≥600? PMV Infection	(Visser 1993, Osterhaus <i>et al.</i> , 1995a)
Lake Baikal seals ( <i>Phoca siberica</i> )	Lake Baikal, Russia	CDV Population crash	(Grachev <i>et al.</i> , 1989)
Caspian Seals ( <i>Phoca caspica</i> )	Caspian Sea	CDV Population crash >1000	(Kennedy <i>et al.</i> , 2000)

In some cases exposure to a morbillivirus has been attributed to transmission from terrestrial or marine species. Opportunities for transmission between terrestrial and aquatic species do exist as exposure to a range of morbilliviruses (one or more of CDV, DMV and PDV) has been demonstrated in a number of terrestrial carnivores known to feed on seals, including polar bears (*Ursus maritimus*) (Cattet *et al.*, 2004, Philippa *et al.*, 2004) domestic dogs (Lucas & Stobo 2000), red foxes (Andriashek & Spencer, 1989, cited in Way 2004) (Little *et al.*, 1998b), coyotes (Gese *et al.*, 1991, Cypher *et al.*, 1998, Arjo *et al.*, 2003, Way & Horton 2004) and black-backed jackals (Oosthuizen *et al.*, 1997a) (this study, Chapter 3).

The cross-species transmission may result in the establishment of the morbillivirus in the spill-over host population. Hence the discovery of PDV and MV (morbillivirus) VN antibodies in polar bears from Canada and Russia respectively (Follmann *et al.*, 1996, Philippa *et al.*, 2004) has led some to speculate, that if the virus is indeed PDV, the most likely source would be the polar bears' prey i.e. seals (Follmann *et al.*, 1996). Follmann *et al.*, (1996) also suggest that the higher seroprevalence of a MV in the polar bears of the Russian Arctic may mean that a MV is endemic in this area. The introduction of CDV into the Antarctic crab-eater seal population, which is now thought to be a maintenance population for this virus (Bengston *et al.*, 1991), is

attributed to domestic dogs (sledge dogs) on the basis of the fact that there are no indigenous terrestrial carnivores in the Antarctic (Forsyth *et al.*, 1998). Transmission of CDV from domestic dogs is also thought to have been responsible for the outbreaks in the Caspian and Lake Baikal seals as both areas support large populations of feral and domestic dogs in which CDV is thought to be endemic (Mamaev *et al.*, 1995, Mamaev *et al.*, 1996, Forsyth *et al.*, 1998).

Inter-specific transmission is also thought to occur between marine species (Osterhaus *et al.*, 1995b, van de Bildt *et al.*, 1999) and this is considered to be the most likely cause of the 1988 European PDV outbreak (Osterhaus *et al.*, 1995b). Serological surveys have demonstrated the widespread exposure of seals in the Arctic and North America to MVs similar to PDV (Table 4-1) and there is evidence for the presence of PDV or a closely related MV in healthy seals from eastern Canada prior to the 1988 outbreak (Ross *et al.*, 1992). The harp seals, in which exposure to a MV similar to PDV has been demonstrated (Dietz *et al.*, 1989a), are thought to have carried PDV over to Europe in a mass migration (Osterhaus *et al.*, 1995b) and PDV is now thought to be endemic in the European harbour seals (Visser *et al.*, 1993b, Osterhaus *et al.*, 1995b). Finally, transmission between cetacean and pinniped populations is also thought to have occurred, resulting in a mass-mortality of monk seals thought to be due to a cetacean MV (DMV) (van de Bildt *et al.*, 2000, van de Bildt *et al.*, 2001) as a mass mortality in dolphins from the same area was observed 1 year before the monk seal mortality (unpublished data, van de Bildt *et al.*, 2001).

Hence this study was motivated by two main concerns a) the possibility that a morbillivirus may be endemic in the Namibian Cape fur seal population and b) the potential for spill-over of CDV from sympatric jackals and domestic dogs on the Namibian coast, which suffered a CD epidemic in 2002-3. Information on the exposure of morbilliviruses such as CDV and PDV is important because a number of factors may interact to exacerbate the effect of morbilliviral infections in a population, resulting in a significant reduction in population size and an increase in the risk of extinction (Van Bresseem *et al.*, 2001). Co-factors may be the additional

mortality from, for example human activities such as harvesting (Van Bressem *et al.*, 1999), or the immunosuppressive effects of pollutants which may make pinnipeds more susceptible to severe disease (Ross *et al.*, 1995, Ross 2002). The Namibian Cape fur seal population has suffered at least two mass-mortalities (Gerber & Hilborn 2001) and excessive harvesting in the 19<sup>th</sup> century drove this population to near extinction (Barnard 1998).

#### **4.2.1 Endemicity of a morbillivirus?**

A survey by Anselmo *et al.* (1995, unpublished data, Appendices Chapter 4) investigated the possibility that an infectious disease, likely a morbillivirus, may have been responsible for (at least in part) the mass mortality experienced by the Cape fur seal population in 1993-4. Accurate estimates of the mortality are not available but it is thought that the mortality affected primarily pups and that most, if not all of the 1993 cohort of approximately 200,000 pups died over the course of 1993-4 (Van Zyl 1999). Anselmo *et al.* (1995) performed a serological survey for exposure of pups, sampled in 1994, to CDV and PDV using a VNT. They reported a high level of exposure to a MV in seal pups (65.2%,  $n=23$ , 95% CI 42.7-83.6). This MV was thought to be either CDV or PDV but the viruses could not be conclusively distinguished from the results of the VNTs. The involvement of a MV in the mass-mortality was questionable as most of the individuals found at the mainland colonies were stranded and moribund pups in very poor condition and none of these showed any clinical signs of infection. Gross *post mortem* examinations ( $n=41$ ) did not detect any lesions or changes consistent with a morbillivirus infection. The absence of clinical signs and pathological changes indicative of a morbillivirus infection, the pronounced emaciation of the dead pups, the absence of adults at the colonies (which are capable of foraging further out at sea than pups), and the failure to isolate (by the cell culture of organ tissue homogenates) any morbillivirus from the tissues of seropositive and seronegative individuals led the investigating team to conclude that starvation was the cause of the mortality and that "no indications were found for a role of an infectious disease in the mortality". It was suggested however, that a

morbillivirus was endemic in this seal population and that an unidentified adenovirus, for which 100% exposure had been detected ( $n=23$ ), was also endemic.

The likely cause of the starvation was the collapse of the local fish stocks due to an anoxic event in the Benguela system (Cochrane 1997, Van Zyl 1999). These events are commonly known as Benguela-Niños and involve the intrusion of warm, low-oxygen content water from the Angolan current in the north which in turn results in severe alterations in the distributions of fish stocks in the Benguela system (Barnard 1998). The 1993-4 mass mortality was not the first to occur in this seal population. Another mass mortality of unknown aetiology occurred in this population in the 1820s (Wyatt 1980) and this is thought to have affected 500,000 seals. But the cause, and the true extent, of this mortality are unknown although starvation due to a Benguela-Niño or biotoxin poisoning from a ‘red tide’ may have been responsible (Gerber & Hilborn 2001).

Investigations of mass mortalities in wildlife are typically very difficult as more than one factor may be responsible. In the case of the Namibian Cape fur seals, two factors, namely a morbillivirus and malnutrition may have been involved. On the basis of the evidence collected by Anselmo *et al.* (1995) the presence of an endemic MV remains doubtful. The small sample size of the Anselmo study, consisting of one sampling year and one age class (pups which may have had maternal antibodies), does not provide definitive evidence for the endemic or epidemic persistence of a morbillivirus in this population, particularly since there are no data on exposure, prior to, or immediately after this mass mortality.

The endemic persistence of morbilliviruses in pinniped populations is a controversial subject. On one hand, one would expect morbilliviruses to persist in large pinniped populations such as the East Atlantic harbour seal population, estimated at 30,000-100,000 animals (Bigg, 1981, cited in Swinton 1997) and the large Cape fur seal population, estimated at over 1 million individuals (Pallett 2000). Indeed, PDV does seem to be persisting in the European seal populations since the 1998 mortality (Osterhaus *et al.*, 1995b) as virus has been detected since then (Visser *et al.*, 1993b).

But epidemiological modelling of PDV in the harbour seals indicated that endemic persistence would not be possible as the CCS was estimated at 100,000,000 animals (Swinton *et al.*, 1997). In conclusion, further evidence in addition to population size and the results of the Anselmo study are required to determine if a morbillivirus is endemic in the Cape fur seal population.

#### 4.2.1.1 *Current mortality*

Each year, an as yet undetermined number of seals at both Cape Cross and the Wolf and Atlas Bays are seen with CNS signs consisting of the paralysis of the fore or hind flippers and seizures, which occur in pups and adults of both sexes (pers. comm. S. Kirkman, Ministry of Fisheries and Marine Resources). Unfortunately no information is available regarding the geographical distribution or the percentage of individuals affected. There are many causes of neurological symptoms in pinnipeds including natural toxins and numerous infectious diseases. Immunosuppression, which itself may be caused by toxins, pollutants or infectious diseases may be a co-factor in the causation of CNS signs.

A review by Harvell *et al.* (1999) highlighted the increasing frequency in the occurrence of toxic marine algal blooms, commonly known as 'red tides' or harmful algal blooms (HABs), which can result in the crash of fish stocks (Harvell *et al.*, 1999). Pinnipeds and other marine mammal predators may suffer damage to the CNS by the ingestion of neurotoxins (e.g. domoic acid) when they feed on contaminated fish or crustaceans (Scholin *et al.*, 2000, Kvitek & Bretz 2004). A mass mortality of Californian sea lions (*Zalophus californianus*) has been attributed to toxins from a red tide and the clinical signs included seizures, ataxia, head weaving and depression (Scholin *et al.*, 2000). It has been debated whether algal toxins played a role in the mass mortality of the Mauritanian monk seals in which a morbillivirus was also thought to be involved (Hernandez *et al.*, 1998, Osterhaus *et al.*, 1998).

The neurological signs may also have a bacterial or viral aetiology. If a morbillivirus were endemic in the Cape fur seal population this may cause CNS signs in infected



individuals, or result in immunosuppression allowing secondary bacterial infections which can also cause CNS signs. Morbilliviruses are known to be immunosuppressive (Heaney *et al.*, 2002) and co-infection with bacteria has been reported in Mediterranean striped dolphin during the 1990-1992 cetacean morbillivirus epizootic (Domingo *et al.*, 1995).

Numerous species of bacteria have been isolated from seals with morbilliviral infections (Tewes, S., 1989 cited in Baker & Ross 1992) (Baker 1992, Baker & Ross 1992, Barrett *et al.*, 2004). Bacteriological examinations of 57 common seals with PDV from the German North Sea coast identified infection (in 45 seals) with  $\beta$ -haemolytic *Streptococcus* spp. (15.8%), *Bordetella bronchiseptica* (12.3%), *Escherichia coli* (7.0%), *Corynebacterium* spp. (7.0%), *Klebsiella* spp. (5.3%) and *Pseudomonas aeruginosa* (3.5%) (Tewes, 1989). In an investigation of the bacterial infections associated with PDV in common seals from British shores, Baker *et al.* (1992) found all of the species isolated by Tewes (1989) including *Klebsiella* spp. (1.4%) in lung tissue samples (Baker & Ross 1992).

A species of *Klebsiella* has also been isolated from Namibian Cape fur seals which were suffering from the CNS signs described above. In an investigation by the CVL into the possible cause of the CNS signs, 7 carcasses or brains of pups from the WAB colony of the Diamond Area (2002) were examined for histopathological changes and tissue samples from all the major organs including the brain were cultured for bacteriology (Dr. F. Mettler and Dr. G. Eberle, unpublished data). Histopathological analyses indicated that the pups suffered from bacterial meningitis whilst pure cultures of 4 of the 7 brain samples, and all organ tissues tested from whole carcasses resulted positive for the *Klebsiella pneumoniae*.

*K. pneumoniae* is an opportunistic gram negative enterobacterium, capable of producing endotoxins. It is commonly found in humans, animals, soil, sawdust and in a variety of marine mammal species (Smith *et al.*, 1978, Quinn & Carter 1994). *Klebsiella* spp. have been reported in the lungs and livers of a variety of pinniped species and been isolated from the brains of stranded seals in California (Johnson *et*



*al.*, 1998, Thornton *et al.*, 1998); the Californian sea lions were also seen to suffer from seizures of an unknown aetiology during two mass strandings (Gerber *et al.*, 1993). Therefore immunosuppression may be a requirement for *Klebsiella* spp. to cause widespread infection and/or disease in seals. One possible cause of this immunosuppression, to be investigated in this study, is a morbilliviral infection.

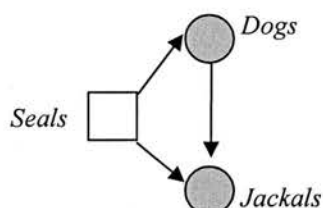
There are, of course, other causes of immunosuppression in addition to infectious diseases. There is extensive evidence that pollution can have an immunosuppressive effect on marine mammals, making them more susceptible to viral and bacterial infections. Organochlorines, particularly polychlorinated biphenyls (PCBs), have been shown to have immunotoxic effects, impairing cellular immune responses in harbour seals and making them potentially more susceptible to morbilliviral infection (De Swart *et al.*, 1995b, Ross *et al.*, 1995, De Swart *et al.*, 1996, Ross *et al.*, 1996a, Ross *et al.*, 1996b). High PCB levels may also have played a role in the morbilliviral-induced mass mortalities in striped dolphins in the Mediterranean (Aguilar & Borrell 1994) and common dolphins (*Delphinus delphis ponticus*) in the Black Sea (Birkun *et al.*, 1999). Elevated PCB levels have also been linked to infectious disease mortality in the UK-stranded harbour porpoises (Jepson *et al.*, 1999). But pollution is not thought to affect the cape fur seals sufficiently as to cause immunosuppression as the desert coast of Namibia is virtually devoid of permanent human settlement and not suitable for agricultural development. As a consequence the normal levels of pollution associated with urban communities, shore-based industries and agriculture do not occur on the Namibian coast (Barnard 1998). Monitoring at Walvis Bay and the coastal mining areas of the Diamond Area indicate that pollution is localised and not a major threat to biodiversity on a large scale (Barnard 1998).

## 4.3 Aims

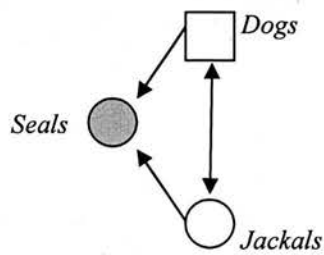
This study uses an extensive serosurvey, the first of its kind for the Cape fur seal, together with a preliminary survey for *Klebseilla* spp., to determine if there is exposure to CDV (endemic or epidemic in nature) in the seal population of Namibia and to investigate the possibility of co-exposure to both CDV and *Klebseilla* spp. The aims of this study and the hypotheses under investigation may therefore be summarised as follows:

1. To determine from serosurveys for CDV if a morbillivirus is endemic in this seal population and therefore whether
  - a. a morbillivirus is likely to be responsible for the mortalities in individuals exhibiting CNS signs,
  - b. the seals were likely to be the source of the virus which caused the epidemic in the jackals and dogs of the coast (Figure 4-1),
  - c. the seals were affected by the CD epidemic in the jackal population (Figure 4-2).
2. To determine the presence of *Klebseilla pneumoniae* in nasal swabs from jackals and seals.

**Figure 4-1: Hypothesis 1- The seal population as the source and maintenance population for the CDV which caused the epidemic in jackals and dogs.** Filled circles indicate target populations and boxes possible maintenance populations (Haydon *et al.*, 2002).



**Figure 4-2: Hypothesis 2- The jackal and dog populations as the source and maintenance populations respectively, for CDV in seals.** As proposed in Chapter 3, dogs are thought to be the maintenance population for CDV and jackals a source of CDV (blank circle); in this hypothesis the seals are the target population (Haydon *et al.*, 2002).



## **4.4 Materials and Methods**

### **4.4.1 Access to the seals**

Permits to sample during the seal harvests at both study sites were obtained from the Ministry of Fisheries and Marine Resources and from the Ministry of Environment and Tourism. The project also liaised with the harvest concessionaires for access during the harvest. Due to the sensitive nature of the harvesting, no recording other than the sample numbers and body measurements was permitted during the harvest which was monitored on site by officers of the Ministry of Fisheries and Marine Resources.

### **4.4.2 Sampling of the Cape fur seals**

#### *4.4.2.1 Study sites and sampling*

Seals were sampled from the two study site colonies, namely the Wolf and Atlas Bays of the Diamond Area and the CCSR as these are the only two colonies at which seal harvesting takes place. Further details of the study site selection are provided in Chapter 2 (sections 2.3.2-4). The sampling was restricted to male and female pups and adult bulls as adult females (cows) are not included in the harvests. Nonetheless, a small number of cows were included in the sampling because they were mistaken for bulls by the culling team. Sampling took place between approximately 06:00 and 10:00am and as many individuals as possible were sampled each morning. The culling team were followed as closely as possible and every animal whose blood had not yet clotted was sampled, the numbers of seals sampled being constrained by the time taken to access the carcasses. Sampling days were selected largely opportunistically but to be as evenly distributed across as much of the 4-and-a-half month harvest period (July to mid-November) as was logistically possible.

#### 4.4.2.2 Sample size

The study by Anselmo *et al.* (Anselmo *et al.*, 1995) revealed a seroprevalence of a morbillivirus of 65.2% ( $n=23$ , 96% CI 42.7-83.6). The approximate sample size required to estimate this seroprevalence in a large population within a 95% confidence interval can be determined by (Thrusfield 1995b):

$$n = \frac{1.96^2 P_{\text{exp}} (1 - P_{\text{exp}})}{d^2} \quad \text{Equation}$$

1.0

where  $n$  is the required sample size,  $P_{\text{exp}}$  the expected proportion of seropositives (0.652) and  $d$  the desired absolute precision which in this case is 5% (i.e. the 95% confidence interval is 60.2% to 70.2%). From these values the required sample size was calculated to be 349 seals and this was set as the target sample size for each sampling year.

The error surrounding seroprevalence estimates, given the population size, expected seroprevalence and sample size, was calculated using the 'absolute error' function of the 'Sample size' section in WinEpiscope 2.0 (Blas *et al.*, 1998). The maximum number of positive individuals (seroprevalence) which could have gone undetected, given the number of negative samples and the population size, was calculated in WinEpiscope 2.0 using the 'Detection of disease' function's 'maximum no. of positives' option. The probability (%) of detecting one exposed individual given the population size, estimated seroprevalence and sample size was calculated using the 'level of confidence' option of the 'Detection of disease' function.

#### 4.4.2.3 Blood samples

Blood for serum was sampled from the open chest cavities of individuals as cardiac puncture is performed by the culling team to ensure death. Between 5 and 10ml of blood was collected in a sterile tube as soon after death (no later than 10 minutes) as possible to ensure that no clotted blood was sampled. All blood samples were

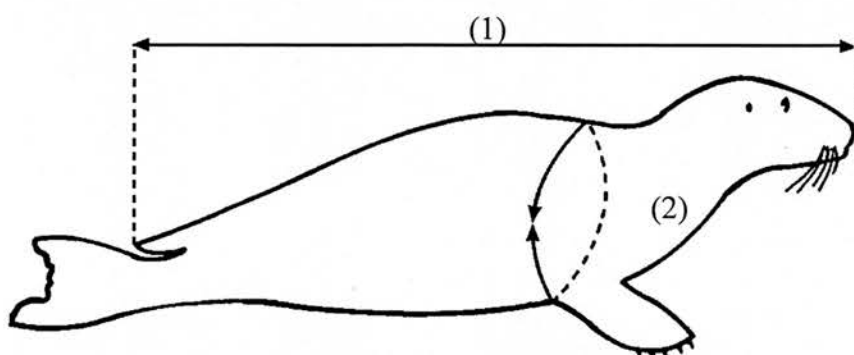
refrigerated, left to clot and centrifuged within 12 hours of collection. Serum samples were stored and heat inactivated prior to testing as described in Chapter 2 (section 2.5.2). Aliquots of each sample were stored with the CVL in Windhoek to provide a reference library for future research and a source of back-up samples.

#### *4.4.2.4 Ageing of seals and additional data*

At the time of sampling, individuals were classed as either pups or adults. Individuals classed as pups were those of one year of age or just under (i.e. those born in November and December prior to the sampling year). Those classed as adults were those over one year of age; adults were easily distinguishable from pups by the difference in size and fur colour. This age classification does not distinguish between juveniles and adults. Juveniles are those over one year of age but which have not yet reached sexual maturity; cows reach sexual maturity between 3 and 6 years of age whilst bulls are sexually mature at 4-5 years (Macdonald 2001).

In order to obtain age estimates in years for the adults sampled in this study, body length measured to the nearest cm (Figure 4-3) was correlated, using survival curves, with age in years. Seals were aged in years from the counts of dentine growth layer groups (GLGs) of adult canines (Oosthuizen & Bester 1997). Point estimates of age in years for males and females were estimated from separate growth curves each sex. The growth curves (courtesy of Dr. H. Oosthuizen of the Directorate Marine and Coastal Management, Cape Town, South Africa and Mr. P. Odendaal, Department of Zoology and Entomology, University of Pretoria, South Africa) were obtained by applying the Gompertz relation ( $\text{body length} = a \cdot \exp(-\exp(b - cx))$  where  $a, b$  and  $c$  are sex-specific constants and  $x$  is age in years) to GLG counts and body length data from 572 females and 562 males sampled between 1971 and 1990 by the Directorate Marine and Coastal Management. Because the pulp cavity of Cape fur seals closes at around 14 years (pers. comm. P. Odendaal), those over 14 years of age could not be aged. The girths of individuals were also measured (Figure 4-3) and the sex determined. All data were submitted to the Ministry of Fisheries; girth data is used as a surrogate measure of condition.

**Figure 4-3: The measurement of body length (1) and girth (2) in pup and adult seals.** Adapted from the Marine Mammal Stranding Project data sheet, Courtesy of P. Jepson, Institute of Zoology, London.



#### 4.4.2.5 Virus neutralisation tests

Due to laboratory and financial constraints not all the seal serum samples collected could be analysed. For CDV, a random selection of approximately 50 pups and 75 bulls from each study colony and sampling year were tested. 25 more bulls than pups, per sampling year and location, were tested because they are older and more likely to be exposed to a virus which may have been circulating in the population. The sample sizes of 50 pups and 75 bulls both gave a 100% probability of detecting one exposed individual assuming a 65.2% or 30% prevalence of exposure in a colony (*c.*190,000 individuals, section 2.3.3) or the Namibian population (*c.*900,000 individuals). The additional bull samples were selected by using body length as an indicator of age *i.e.* 25 bulls with the longest body lengths. If one or more samples from a particular age class, sampling year and location resulted positive, additional samples from the same subset were selected at random for testing.

VNTs for CDV, CAV-1 and canine herpesvirus (CHV) were performed at the Intervet laboratories (UK), essentially as described for the jackal and dog sera in Chapters 3 and 5 (sections 3.3.7 and 5.3.3). But in the case of CDV only two serum dilutions (1:8 and 1:32) were tested (to facilitate the processing of large numbers of samples) and in the case of CHV all 4 serum dilutions were used. Because phocine herpesvirus type 1 (PhHV-1) is a common infection in seals and there is cross reaction between herpesviruses of terrestrial and aquatic carnivores (Greene 1998,



Gaskell & Willoughby 1999, Goldstein *et al.*, 2003), the tests for CHV were performed to check only for the presence of antibodies as a control for the quality of the serum samples. The tests for CAV-1 in the seals were performed to investigate the potential for exposure of seals to an adenovirus from jackals, as well as to ascertain the quality of the serum samples as antibodies to this virus had previously been detected in this population (Anselmo *et al.*, 1995). Samples tested for CAV-1 and CHV were selected at random from the subset tested for CDV and were only recorded as positive or negative for VN antibodies (no titre calculated and no cut-off point applied) on the presence or absence of a cytopathic effect respectively. Any samples which resulted positive for CDV were re-tested using all 4 serum dilutions and a titre calculated; a cut-off of  $\geq 20$  was used to minimise the likelihood of false positives occurring as a result of cytotoxicity of the sera, also encountered in other serosurveys (Muller *et al.*, 2000).

In order to allow a direct comparison with the results of the Anselmo study and to verify the results of the CDV VNTs performed at Intervet, VNTs for CDV were also performed by Dr. M. van de Bildt (Department of Virology, Erasmus University, the Netherlands) on 167 samples, selected at random from the subset tested at Intervet. The samples were tested blind and the VNTs performed with CDV Bussel strain as per Visser *et al.*, (1990). In addition the same 167 samples were tested using the same VNT methodology for antibodies to PDV (PDV/1/88NL) and DMV (DMV 16a). In the case of CDV, only those samples which tested positive at both Intervet and Erasmus University were considered positive. If any samples were seropositive for CDV these were also tested for PDV to ascertain which virus the individuals were exposed to as there is significant cross-reaction between antibodies to these two closely related morbilliviruses (Cornwell *et al.*, 1992, Saliki *et al.*, 2002, Stanton *et al.*, 2004). For each individual, the positive titres from each test were compared and the antibodies were assumed to be directed against the virus whose test had the highest titre.

#### 4.4.2.6 Nasal swabs

Swabs were collected from jackals as described in Chapter 2. Nasal swabs were taken as *Klebsiella* spp. are known to occur in the lungs of seals and are therefore likely to be found in the respiratory passages of seals (Baker & Ross 1992, Thornton *et al.*, 1998), and possibly jackals. Sterile swabs were gently inserted into the nasal passages of dead seals and jackals which had not bled out of the nasal passages, and from captured jackals under sedation; care was taken not to touch the swab to any surface other than the nasal passages. Only adult seals were sampled as it was not possible to swab pups as the harvest had fulfilled the pup quota by the time of sampling. All swabs were sterile, refrigerated after collection and cultured by Dr. G. Eberle, head of Microbiology, CVL (Windhoek).

## 4.5 Results

### 4.5.1 Sample size

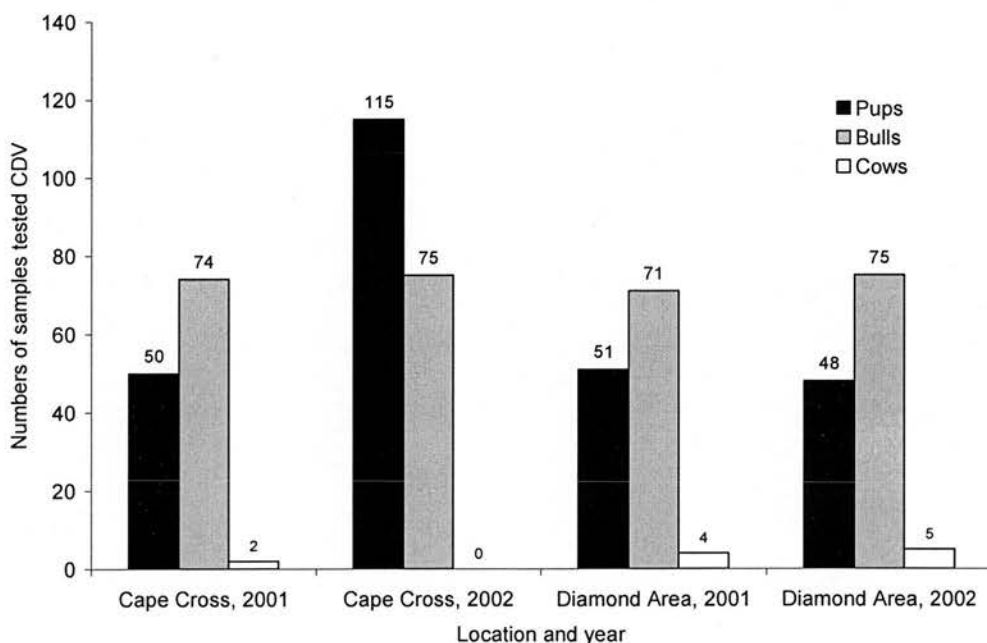
The target sample size of 349 was achieved in both sampling years with a total of 380 and 887 seals sampled in 2001 and 2002 respectively ( $n=1267$ ; 589 pups, 667 bulls and 11 cows). Unfortunately, only 45% ( $n=570$ ) of the total sample set could be tested. A larger field team in 2002 made it possible to greatly increase the sample sizes at both study sites.

### 4.5.2 Serosurvey

#### CDV

A total of 570 serum samples were tested for antibodies to CDV and the sample sizes for each study colony are given in Figure 4-4.

**Figure 4-4: The numbers of pups, bulls and cows tested for VN antibodies to CDV.  $n=570$  with sample sizes given above each bar.**



Of the serum samples which were tested at Intervet 5% showed a cytopathic or cytotoxic effect on the Vero cells during incubation. Overall seroprevalences of 0.19% ( $n=535$ , 95% CI 0.0-1.0) and 0.18% ( $n=570$ , 95% CI 0.0-1.0) were calculated excluding and including the cytotoxic samples as negative sera, respectively. Only one individual tested seropositive at Intervet– a pup from Cape Cross sampled in 2002 – with a titre of 1:161. The resulting seroprevalences in pups at Cape Cross in 2002, including and excluding 3 samples which resulted toxic were 0.87% (95% CI 0.0-4.8) and 0.89% ( $n=112$ , 95% CI 0.0-4.9).

Of the 167 samples tested for CDV at Erasmus University a similar percentage (6%) showed cytotoxic effects. Two individuals resulted seropositive to CDV in the tests performed at Erasmus University – the pup from Cape Cross and a bull from Lüderitz also sampled in 2002. The seroprevalence excluding cytotoxic samples ( $n=10$ ) was 1.2% ( $n=150$ , 95% CI 0.2-4.3) and excluding these samples the seroprevalence was 1.3% ( $n=167$ , 95% CI 0.2-4.5).

#### *PDV and DMV*

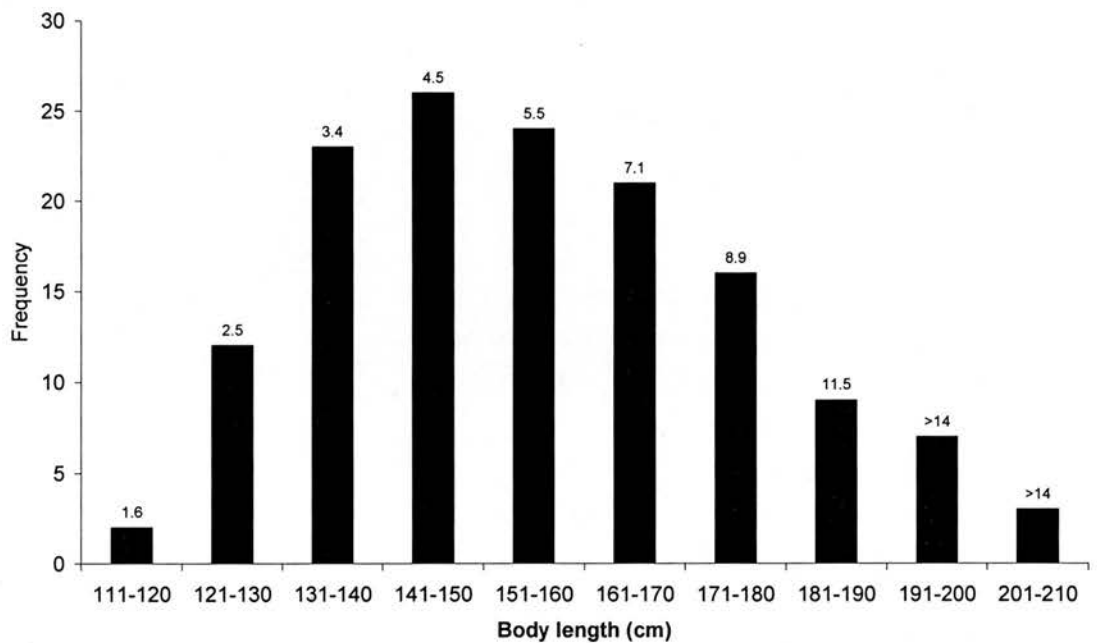
None of the seals tested seropositive to DMV or PDV (0.0%,  $n=167$ , 95% CI 0.0-2.2), including the pup and bull which were seropositive to CDV.

#### *CAV-1 and CHV*

Of the 80 individuals tested for CAV-1, 22.5% (95% CI 13.9-33.2) were seropositive; no toxic effects were detected in the MDCK cells used for these tests. The A72 cells used for the CHV VNTs were far more sensitive to the toxic effect of the sera (~50% showed toxic effects at the lower dilution of 1:8) and so for those cultures showing toxic effects only those samples which showed clear viral-induced cytopathic effects in the top two dilutions were considered positive. The resulting seroprevalence was 11.4% ( $n=290$ , 95% CI 8.0-15.6).

It was not possible to record body measurements and sex for all individuals as sampling had to be rapid in order to obtain un-clotted blood. The age distribution of the bulls used in the serosurvey is shown in Figure 4-5 with point age estimates in years for the midpoint of each body length category. Body length measurements were available for 10 of the 11 females tested. Two females were over 14 years of age (>145cm), the ages of the remaining females ( $n=8$ ) ranged between 1.9 and 14.0 years; a total of 5 females were over 8 years old.

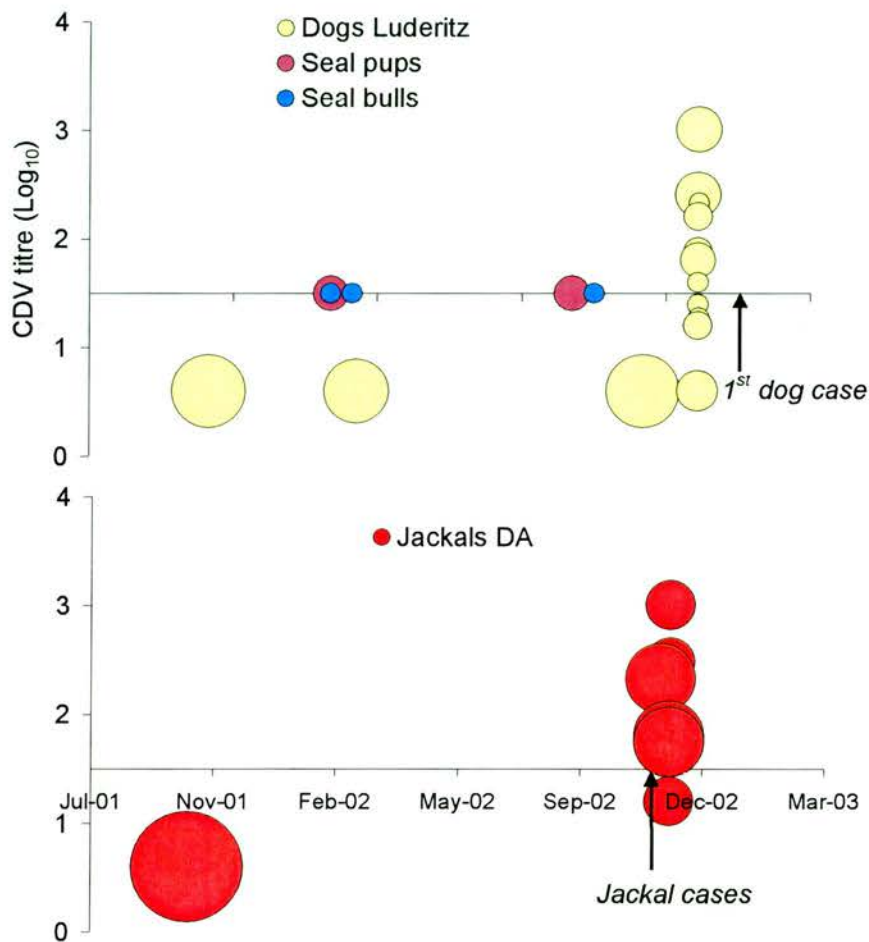
**Figure 4-5: The frequency distribution of the body lengths of seal bulls tested in the CDV serosurvey.  $n=143$ .** Age estimates in years for the midpoint of each body length class are given above each bar; those over 14 year of age could not be aged.



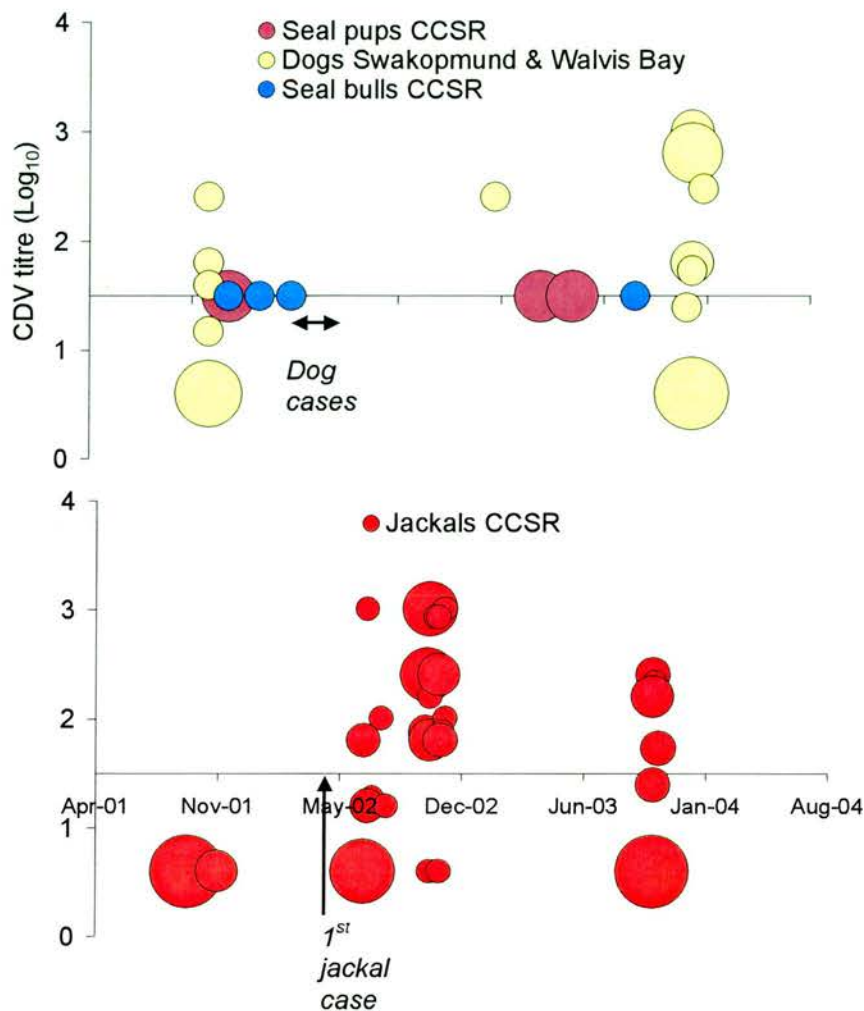
Figures 4-6 and 4-7 show the sampling of the seals with respect to the CDV outbreak in jackals and dogs (as determined from the serosurvey in 2002-3) for the southern and northern study sites respectively. In 2002, seals in the Diamond Area were sampled before the detection of clinical signs of distemper infection in the jackals of that area (November) and well before the first suspected case in dogs (end December). The seals at Cape Cross were sampled after the start of the CDV outbreak in jackals. As described in Chapter 3 (section 3.4.1) the index case in jackals at Cape Cross is thought to have occurred at the end of April 2002 (vertical

arrow Figure 4-7); the peak of the epidemic in dogs is thought to have occurred between April and May (horizontal arrow Figure 4-7).

**Figure 4-6: The sampling of the seals which were tested for antibodies to CDV with respect to the occurrence of CDV seropositive jackals and dogs at the southern study town and seal colony, 2001-2003. In jackals and dogs a titre ( $\log_{10}$ ) of  $\geq 1.5$  is considered positive. The cut-off point is indicated by the x axis.**



**Figure 4-7: The sampling of the seals which were tested for antibodies to CDV with respect to the occurrence of CDV seropositive jackals and dogs at the northern study towns and seal colony, 2001-2003. In jackals and dogs a titre ( $\log_{10}$ ) of  $\geq 1.5$  is considered positive. The cut-off point is indicated by the x axis.**



### 4.5.3 Observed morbidity and mortality

Each year at both study colonies, a small and as yet undetermined number of adults and pups (no information was available from the Ministry of Fisheries at the time of writing) are observed with clinical signs which comprise paralysis of the hind flippers and full body seizures. In 2002 an adult cow was seen to haul-out alone on the beach approximately 2km from the main colony at Cape Cross. This individual was in poor condition, but not emaciated, and suffered a number of seizures prior to death. Blood from the heart was sampled for testing. This individual was seronegative to CDV. One other individual, a pup at Cape Cross, was seen in 2003



with similar signs but it was not possible to observe this individual to determine if it died or to retrieve diagnostic material. In 2002-3 no increase in mortality was reported at either study colony by the officers of the Ministry of Fisheries working at colonies along the coast, before, during or after the CD outbreak in jackals and dogs of the coast (pers. comm. Ministry of Fisheries).

#### **4.5.4 Nasal swabs**

None of the adult seals ( $n=40$ ) or jackals ( $n=20$ ) resulted positive for *Klebsiella* spp. (pers. comm. Dr. G. Eberle, CVL). Seal pups could not be sampled as the harvest had fulfilled the pup quota by the time the nasal swabs were taken.

## **4.6 Discussion and conclusions**

### **4.6.1 Is a morbillivirus endemic in the Namibian Cape fur seal population?**

Several lines of evidence indicate that neither CDV nor PDV is endemic in the Namibian cape fur seal population. Firstly, the seroprevalence of CDV in 2001 was zero and only one seropositive was detected in 2002. Secondly, none of the individuals tested resulted seropositive to PDV or DMV. This conclusion is supported by the absence of exposure in the older animals which would have been alive at the time of the 1994-5 mass mortality i.e. any individuals of  $\geq 8$  years of age ( $n=35$  bulls and 5 cows).

Several factors need to be considered as artefactual causes of the low seroprevalence. Firstly, the low seroprevalence may be attributed to the poor quality of the serum samples as these were collected under non-sterile conditions (free-flowing blood from the body cavity collected using open tubes) and subject to more than one freeze-thaw cycle before testing. These conditions may act to destroy antibodies present in the samples thus reducing any positive titres to negative titres or undetectable levels. But this is unlikely to be the case as 22.5% (95% CI 13.9-33.2) and 11.4% (95% CI 8.0-15.6) were positive for VN antibodies to CAV-1 and CHV respectively. The presence of detectable VN antibodies from viruses other than CDV indicates that the sera were not negative to CDV because of the degradation of the antibodies. It is possible however, that some of the CDV-seropositive samples may have not been detected because of the occurrence of a cytopathic effect on the cells during incubation. But the low prevalence of this effect (5-6%) in such a large sample size is unlikely to result in any qualitative changes in the conclusions drawn from the results of these analyses, if the status of the toxic samples were known. Indeed, the 95% confidence intervals of the seroprevalence estimates with (95% CI 0.0-4.8) and without (95% CI 0.0-4.9) the toxic samples overlap almost completely. No conclusions are drawn here regarding the adenovirus and herpesvirus strains circulating in this seal population as there is serological cross-reaction between herpesviruses of terrestrial and aquatic mammals (Gaskell & Willoughby 1999) and

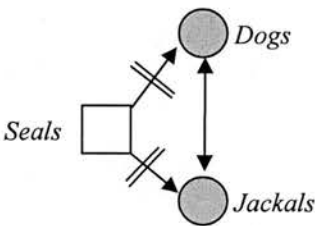
there is likely to be cross-reaction between adenoviruses which are highly conserved (Greene 1998) and for which antibodies have been detected in other marine mammals (Smith & Skilling 1979, Dierauf *et al.*, 1981, Philippa *et al.*, 2004).

The use of positive and negative controls with each batch of samples in the VNTs and the calculation of the TCID<sub>50</sub> for each test ensured that the VNTs were working. Therefore the results were not due to a poor quality test. The low seroprevalence may also be attributed to the absence of a detectable immune response after exposure. But the Cape fur seal, like other pinnipeds species (Cornwell *et al.*, 1992, De Swart *et al.*, 1995a, Philippa *et al.*, 2004), is capable of mounting an immune response and producing detectable antibodies to morbilliviruses as evidenced by the seropositive pup in this study and the seropositive individuals in the study by Anselmo *et al.* (Anselmo *et al.*, 1995). Furthermore, antibody responses to morbilliviruses are thought to be life-long (Harder *et al.*, 1990, Visser 1993). Hence the negative result is unlikely to be due to the absence of an immune response.

It is unlikely that the seroprevalence estimated by Anselmo *et al.* (1995) went undetected. A total of 345 individuals were required to detect a seroprevalence of 65.2% (Anselmo *et al.*, 1995) within a 95% confidence interval of 60.2%-70.2% and over 500 seals were tested. The testing of 312 and 254 seals from Cape Cross and the Diamond Area respectively allows for the estimation of the seroprevalence (65.2%) within reasonable confidence limits (59.9-70.5% for Cape Cross and 59.4-71.1% for the Diamond Area). The sample size of the older animals ( $n=40$ ) allows for a 100% probability of detecting one exposed individual in a population of 900,000 individuals and therefore confidence can be placed in the result of zero seroprevalence in older seals. But if the seroprevalence in the population were a lot lower than 65.2% far larger sample sizes would be required than possible in this study as a maximum of 1792 and 2227 individuals may have gone undetected at Cape Cross and the Diamond Area respectively.

In light of the evidence considered above, it is considered unlikely that the seals were the maintenance population for, or the source of, the canine distemper epidemic in the jackals and dogs of the coast (Figure 4-8).

**Figure 4-8: The Cape fur seals were not the source of the CD epidemic which affected jackals and dogs in 2002-3.**



One may expect, given the size of the Cape fur seal population that a morbillivirus could persist endemically once introduced. But without disease and exposure data from the years preceding or the years immediately after the mass mortality, and the possibility that the antibodies detected in 1994 were of maternal origin (the majority of individuals sampled were pups), it is not possible to say for how long the morbillivirus detected by Anselmo *et al.*(1995) persisted in this population. Suffice to say that none of the animals born since the epidemic tested seropositive, implying that the persistence in the population after the 1993-4 mortality may have been brief. The fluctuation in the prevalence of exposure in this population is more consistent with an epidemic persistence, possibly with fade-out between epidemics, than an endemic persistence.

But it is very difficult to explain the epidemiology behind the negative seroprevalence result of this study given such a high seroprevalence (65.5%) in 1994. It is hard to believe that all of the individuals exposed in 1993-4 mass mortality would have died and therefore been excluded from this serosurvey. Indeed, most of those present at the colonies at the time of sampling were pups, most of which are thought to have died (Van Zyl 1999), but there were also adults present at the colonies and if the pups' antibodies were of maternal origin it follows that adults were exposed. There is evidence that pups did survive the mass mortality as this data set included individuals of 8-9 years of age. Hence if exposure was as high as 65%

then a prevalence higher than 0.2% should have been detected in this serosurvey. The results of this study do shed doubt on the results obtained by Anselmo *et al.* (1995). Ideally the Anselmo study would have performed VNT tests for DMV for further clarification as to allow comparisons of the CDV and PDV titres with a third morbillivirus (Philippa *et al.*, 2004). It may also be that the viruses used in the VNTs were neutralised by something other than MV antibodies. With the absence of exposure in this study it is doubted that CDV or PDV did ever enter this population and establish itself endemically.

#### 4.6.1.1 Possible causes of the observed morbidity and mortality

It was hypothesised that a morbillivirus may have exacerbated infection with *K. pneumoniae* by suppressing the immune response and so allowing the development of bacterial meningitis. The absence of evidence for exposure to a morbillivirus in this study period suggests that the morbidity and mortality observed at the mainland colonies each year is not due to a morbillivirus and that a morbillivirus(es) did not contribute as co-factor(s) to other causes of death, such as *K. pneumoniae* infection. These conclusions are supported by the fact that the symptomatic cow suffering from convulsions sampled at Cape cross in 2002 was seronegative to CDV. It is important to note that the only clinical signs (observed in this case and reported for the pups examined by the CVL) consistent with a morbillivirus infection were the neurological signs. No other signs were observed, including no ocular and nasal discharge characteristic of CDV infection in other seal species (Kennedy *et al.*, 2000).

Assuming that immunosuppression is necessary for *K. pneumoniae* to cause the observed morbidity and mortality it is possible that biotoxins and/or starvation may be the cause of the immunosuppression in the Cape fur seal pups. Further sampling of seal pups, which was not possible in 2002, will be necessary in order to further investigate the occurrence of this pathogen and its role in the observed mortalities. There are two possible explanations for the absence of *Klebsiella* spp. in the adult seals sampled, assuming a high sensitivity and specificity of the culture methods

used in the analysis of the nasal swabs (Dr. G. Eberle, CVL). The first is that all but one of the seals sampled were adult bulls and these would not have had much opportunity for exposure as they were sampled soon after arrival at the colony in October. The alternative explanation is that the sample size was not sufficient to detect *K. pneumoniae* infection in this seal population. If, as in the British common seal population (Baker & Ross 1992), *K. pneumoniae* infection occurs at a very low prevalence (1.4%), this would mean that 213 samples (assuming at total colony size of 187,000 individuals) would be necessary to detect infection and only 40 were tested in this study.

Further surveys for *Klebsiella* spp. in this seal population should be based on the culture of pure tissue samples (such as lung and liver), in addition to the nasal swabs, as *Klebsiella* spp. may not be normally found in the nasal passages of the seals until they are close to death. The identification of the cause of the immunosuppression will require investigations which can link the existence of higher than average biotoxin levels, or starvation, with *K. pneumoniae* infection. Such studies have been performed for harbour porpoises (Jepson *et al.*, 1999, Bennett *et al.*, 2001) and for harbour seals (De Swart *et al.*, 1996). Evidence of impaired immunity will also require analyses of immunological parameters in the blood (De Swart *et al.*, 1995b) and more detailed information on the condition of affected individuals as compared to healthy seals. *K. pneumoniae* can infect dogs (Quinn & Carter 1994) and other canids such as jackals may also be susceptible but all nasal swabs from jackals in this study resulted negative. Certainly, *K. pneumoniae* was not the cause of the neurological signs observed in the jackals.

#### 4.6.1.2 Spill-over of CDV from jackals to seals?

The one seropositive pup in 2002 does not provide unequivocal evidence for spill-over from jackals to seals because it is highly likely that the antibodies in this individual were of maternal origin and it is not possible to determine when and where this cow was exposed. The pup was sampled in August 2002 and would have been approximately 8 months of age. Since lactation in Cape fur seals may last 12

months or more (Macdonald 2001) it is highly likely that the pup was still suckling at the time of the harvest.

The absence of widespread exposure at Cape Cross in 2002 suggests a number of possibilities. The first is that there was no opportunity for transmission between infective jackals and susceptible seals. This is considered to be highly unlikely for a number of reasons. The jackal population suffered high levels of exposure and mortality to CD and over two thirds of jackals captured at this location had clinical symptom(s) consistent with CDV infection (section 3.5.1). This indicates the existence of a large infective pool of jackals over the course of the outbreak in 2002. Symptomatic jackals were observed foraging amongst the seals during behavioural observations (Chapter 6) and one individual with neurological signs was seen to die amongst the seals (pers. obs. S. Gowtage). CDV is a highly contagious virus normally transmitted via aerosol (Greene & Appel 1998) and aerosol contacts between jackals and seals would have been highly likely, particularly since the adult seals do not avoid the jackals and will not move away when attacked (pers. obs. S. Gowtage).

The opportunities for inter-specific transmission may have been limited if CDV significantly altered the behaviour of infected jackals as to reduce the time spent in the colony, for example if infected individuals were less likely to forage at the colony. A number of jackals which were euthanased were often sighted around the buildings in the Cape Cross reserve, over 1km from the seal colony, and these were disorientated and lethargic. Inter-specific contact rates are investigated and discussed further in Chapter 6 and for the purposes of this study, it is assumed that there were opportunities for the transmission of CDV from jackals to seals as the infective period in dogs lasts an average of 13.6 days (Chapter 3, section 3.3.10.1) and there is a period of approximately a week between the start of the infective period and the onset of clinical symptoms (Greene & Appel 1998).

Considering the above evidence, the exposure of the population to CDV may not have been detected if a) the sampling took place too soon after the introduction of the



virus into the seal colony, b) too small a sample size was tested or alternatively, c) CDV failed to spread through the population.

The index case in jackals at Cape Cross occurred towards the end of April 2002 and the pups were sampled between the 29<sup>th</sup> of July and the 8<sup>th</sup> of August. Hence the pups were sampled at least one month after the start the jackal epidemic (the index jackal case was shot at the end of April). Assuming that CDV in Cape fur seals has a similar latent and infectious periods as phocine distemper in harbour seals, of  $\pm 7$  and  $\pm 6$  days respectively (Swinton *et al.*, 1997), the interval between the start or even the peak of the epidemic in July (as detailed in section 3.4.1) would have provided ample time for the spread of the virus through the seal colony. Morbilliviruses are usually spread via aerosol and are considered highly infectious. Aerosol transmission cannot cover large distances but seals at the colonies occur at a very high density which is independent of the colony size (De Koeijer *et al.*, 1998) with cows, pups and bulls often at less than 1m distance from one another (pers. obs. S. Gowtage), which would greatly facilitate transmission.

Age and sex-related differences in haul-out behaviour affect transmission probabilities and rates (Harkonen & Harding 1999, Harding *et al.*, 2002). This is because the likelihood of transmission of a morbillivirus is greater on land due to the aerosol route of transmission (Anderson & May 1982, Hall *et al.*, 1992). The longer time spent at the colony the greater the opportunity for contacts with infective jackals and therefore the transmission of the disease. Hence one of the limitations of this serosurvey is the low numbers of adult cows sampled. These individuals spend relatively much more time on land, suckling pups, than do the bulls which haul-out in October, for the start of the mating season, and leave by the end of December or early January (Pallett 2000, Macdonald 2001). Hence the bulls sampled at Cape Cross in 2002 would not have had much opportunity to be exposed to the virus having spent only a short time at the colony prior to removal by harvesting.

If CDV had spilled-over into the seal population and spread between seals a rapid increase in seroprevalence would have occurred and one may have expected a

seroprevalence of  $\geq 30\%$ , based on the seroprevalence attained in lion epidemics (Roelke-Parker *et al.*, 1996). Assuming that bulls would not have had sufficient time to be exposed, the detection of exposure in a pup cohort of approximately 37,000 individuals (Mukapuli 2004) would have required 9 samples. The sample size of 115 pups allows a reasonable 95% confidence interval for the estimation of a seroprevalence of 30% (95% CI 21.6-38.4). Hence confidence can be placed in the estimate of seroprevalence in pups (0.9%,  $n=115$ , 95% CI 0.0-4.8).

Finally, it is proposed that the strain of CDV which infected the jackals was not capable of spreading between seals, even though it may have infected a limited number of seals as a result of jackal-seal contact. The hemagglutinin protein which sits in the lipoprotein envelope of CDV (Greene & Appel 1998) is a product of the H gene which mediates the attachment of the virus to the host cells and is a determinant of host range and pathogenicity (Carpenter *et al.*, 1998). When the isolate responsible for the mass mortality in the lions of the Serengeti was compared to CDV from other geographic locations, variation in the H gene was detected (Carpenter *et al.*, 1998). Mutations in the H gene may explain why exposure of the lions of the Serengeti region to CDV only resulted in high mortality in 1994, as this population had been exposed to CDV prior to the 1994 mass mortality (Cleaveland, Packer & Lembo, unpublished data). Hence the strain responsible for the epidemic in jackals may lack the necessary changes to H gene (and possibly other genes) necessary to infect, and be transmitted from, (Cape fur) seals.

Spill-over from terrestrial carnivores to sympatric pinniped populations does not always occur. A case in point is the CD epidemic which occurred in the domestic dogs of Galápagos islands in 2001. A CD epidemic swept through the dog populations of the Santa Cruz and Isabela islands in February and March with an estimated peak mortality of 70% (CDF 2001). By the time a state of emergency was declared on the island of Santa Cruz, 50% of the island's population was thought to have been affected with over 150 deaths attributed to CDV (CDF 2001). Fortunately however, despite the widespread mortality in dogs, the Galápagos sea lion (*Zalophus californianus wollebacki*) population was not affected with 35 individuals testing

negative to CDV in March 2001 and with no reports of unusually high morbidity and mortality (CDF 2001).

#### 4.6.1.3 *Future considerations and conclusions*

Canine distemper did not pose a clear threat to the seal population of Namibia at the time of sampling and there was no evidence that this virus played a significant role in the mass mortality in 1993-4. But the presence of seropositive individuals in 1994 and 2002 indicates that there is exposure to a morbillivirus and if this virus were to undergo changes in virulence, the population may suffer increased mortality as result. The inter-specific transmission of morbilliviruses may allow for the evolution of more virulent virus strains (Arya 2000). The CDV strain circulating in the Serengeti, for example, is thought to be a derived strain which is capable of infecting several different species (Carpenter *et al.*, 1998). There are likely to be numerous opportunities for inter-specific transmission in the Namibian and South African region due to the great abundance and diversity of marine life in this region (Barnard 1998).

The jackal population of the coast may not be the only source of CDV for the Cape fur seal population. Until recently dog rescue centres in Cape Town, South Africa, were known to house stranded seals for rehabilitation in close proximity to, and in facilities used for, domestic dogs (pers. comm. S. Kirkman, University of Cape Town). It is thought that over 500 seals were housed in such conditions between 1999 and 2002 (pers. comm. F. Hugo, Seal Alert, South Africa) and in such an environment the potential for direct and indirect exposure (e.g. indirect exposure via feed-preparation areas (Barrett *et al.*, 2004)) to canine distemper, and other infections commonly harboured by domestic dogs, is high. The seals taken into rehabilitation centres are more likely to be in poor condition and therefore immunosuppressed and more susceptible to infection; the release of these individuals back into the seal population may expose other seals to infections acquired whilst in rehabilitation. In addition, the changing distribution of the seal population may affect the risk of pathogen spill-over. The seals are thought to be moving further north into Angolan

waters (Cochrane 1997) and the formation of new mainland colonies could result in increased exposure to canid pathogens.

The highly productive Benguela system supports a wide range of marine fauna including dolphins and whales (Cochrane 1997, Barnard 1998). Vagrant members of two other seal species, namely the Southern elephant seals (*Mirounga leonine*) and the Antarctic fur seal (*Arctocephalus gazelle*), which originate from the Antarctic convergence, also haul out onto Cape fur seal colonies of the Namibian and South African shores (Barnard 1998). These species may act as sources of MVs for the Cape fur seal populations of Namibia and South Africa, particularly if they come into contact with crab-eater seals as CDV is thought to be endemic in this population (Bengston *et al.*, 1991, Forsyth *et al.*, 1998). The exposure of the elephant and Antarctic fur seals to morbilliviruses is unknown but contact with Crab-eater seals cannot be ruled-out. Long distances are not thought to inhibit the spread of morbilliviruses in the marine environment as PDV and DMV have been known to spread through seal and dolphin populations at over 3000 km per year (McCallum *et al.*, 2003). The long range movements of migrant harp seals have been implicated in the spread of PDV from North American to European waters (Ross *et al.*, 1992) and the movements of seals for the transmission of CDV to terrestrial carnivores (Maes *et al.*, 2003). The current range of the Cape fur seals is unknown but tagged individuals have been recovered close to 2000km from the original tagging site and a vagrant Cape fur seal was once sighted approximately 2000km from Cape Town (Oosthuizen 1991). In order to obtain further information on the movements of this species a satellite tracking project, with the satellite tagging of adults from Cape Cross and the Diamond Area, was initiated in 2002 (pers. comm. N. Mukapuli, Ministry of Fisheries).

Continued serosurveillance for morbilliviruses in the Namibia seal population is recommended as the introduction of a morbillivirus in this population may result in a serious population decline. The sampling could be expanded to include other species of marine mammals commonly found in the Namibian waters. The opportunistic sampling of dolphins and other species caught as by-catch (Barnard 1998) on the

numerous fishing vessels operating in the Namibian waters, may provide a good indication of the exposure to morbilliviruses in a number of species; these vessels are regularly patrolled by Ministry of Fisheries officials, who could perform basic sampling for serum and tissues.

Alternative diagnostic tests should also be considered, such as an antigen capture ELISA (Visser *et al.*, 1990), the results of which may not be affected by the cytotoxic effect of the sera as higher dilutions of small quantities of serum are used, and which are also more practical than VNTs which require cell culture facilities and long periods of incubation (von Messling *et al.*, 1999, Saliki & Lehenbauer 2001, Soma *et al.*, 2001). Attempts could be made to detect morbilliviral antigens in tissues using an RT-PCR (Frisk *et al.*, 1999, Kim *et al.*, 2001, Saliki & Lehenbauer 2001, Stanton *et al.*, 2002, Stanton *et al.*, 2004) to determine if, and if so which, morbillivirus is circulating in this population. Tests which detect virus antigens may also be more sensitive than the VNTs in cases where the animal was recently infected as detectable VN antibody levels do not develop immediately upon infection and in cases of severe infection where the individual does not mount a detectable immune response (Soma *et al.*, 2003).

## **Chapter 5: Generalist canid pathogens in dogs and jackals**

## 5.1 Abstract

Serosurveys from a range of ecosystems have shown that jackals, which are sympatric with a wide range of wild species, are exposed to many of the pathogens commonly found in domestic dogs. This study investigated the patterns of exposure to CAV-1, CPV-2, and sarcoptic mange in black-backed jackals and dogs in the coastal region of Namibia to determine if these infections were epidemic or endemic in these species. The jackals and dogs were also surveyed for exposure to CHV and the jackals for helminth infections. The occurrence of sarcoptic mange in jackals is also described and used to evaluate an ELISA for the detection of exposure to *sarcoptes scabiei*. The jackals and dogs sampled between 2001 and 2003 were exposed to all of the pathogens with a high proportion of each species seropositive to 2 or more pathogens (jackals: 93.3%  $n=45$ , 95% CI 81.7-98.6; dogs: 92.9%  $n=42$ , 95% CI 80.5-98.5). The results suggest that sarcoptic mange and CAV-1 are endemic in both dogs (mange: 74.7%,  $n=91$ , 95% CI 64.5-83.3; CAV-1: 77.5%,  $n=80$ , 95% CI 66.8-86.1) and jackals (mange: 78.9%,  $n=71$ , 95% CI 67.6-87.7; CAV-1: 94.3%,  $n=88$ , 95% CI 87.2-98.1) but that CPV-2 is only endemic in the dog population (65.6%,  $n=90$ , 95% CI 54.8-75.3); there is a very low level of exposure to CPV-2 in the jackal population (1.1%,  $n=90$ , 0.0-0.60). The pathology of sarcoptic mange infection in jackals is very similar to that of dogs and the ELISA is a useful tool in the diagnosis of sarcoptic mange in jackals (sensitivity 95.9%, specificity 61.1%). There was a high prevalence of helminth eggs and protozoan oocysts in the jackal population (74.1%,  $n=35$ , 95% CI 53.7-85.4). The jackals and dogs in this study shared exposure to all of the viral pathogens investigated, with the exception of CPV-2. Jackals are likely a source of re-infection for the dog population and are capable of spreading a variety of multi-host canid pathogens well beyond the boundaries of the urban localities.



## 5.2 Introduction

This chapter presents a ‘disease profile’ of the jackals of the Namibian coast which includes the first serosurvey of jackals in Namibia. As discussed in Chapter 1, exposure to multiple canid pathogens in jackals is very common, sometimes with very high prevalences. Although the majority of serosurveys do not provide evidence of actual infection and the pathology of the majority of canid diseases in jackals is unknown, it is likely that the same pathogens cause similar pathogenic effects in jackals and dogs because of their close phylogenetic relationship (Xiaoming *et al.*, 2004).

Jackals in commercial farming areas of Zimbabwe are thought to sustain rabies outbreaks (Bingham *et al.*, 1999) and the same situation is likely in Namibia where outbreaks in black-backed jackals in the central stock ranching area precede those in dogs (Courtin *et al.*, 2000). But there have been no published surveys of pathogens (other than rabies) in jackals in Namibia and so it is not known if other generalist canid pathogens persist in this species.

Aside from CDV, the main diseases of dogs in Namibia, listed by order of importance, are CPV-2, canine ehrlichiosis (*Ehrlichia canis*), canine babesiosis (*Babesia canis*) and Hepatozoonosis (*Hepatozoon* spp.) (Schneider 1994). Other than rabies, cases of sarcoptic mange and anthrax were also recorded in black-backed jackals (Schneider 1994); but it is not clear how the above infections were diagnosed and how prevalent they are in the dog and jackal populations.

CPV-2 is endemic in domestic dog populations worldwide, its ability to persist in the environment for long periods of time greatly increasing the chances of establishment as an endemic infection and of spill-over to wildlife. CPV-2 can cause significant mortality in naïve populations of dogs of all ages but once endemic, mortality is only normally seen in young dogs. The reduction in recruitment, thought to occur African wild dogs and grey wolves (Mech & Goyal 1995, Creel *et al.*, 1997, Creel & Creel 1998) may threaten population persistence if the population is small and vulnerable.

Furthermore, large cats are also susceptible to infection with CPV-2 as the evolution of its two antigenic variants (CPV-2a and CPV-2b) resulted in infectivity for large felids (Ikeda *et al.*, 2002).

Sarcoptic mange is a highly contagious skin disease of mammals caused by the mite *Sarcoptes scabiei* which is spread by direct contact. Mange in dogs and foxes is caused by *Sarcoptes scabiei* var. *canis* and var. *vulpes* respectively (Bornstein *et al.*, 2001) and infection in dogs occurs throughout the world (Muller *et al.*, 2001). The spill-over of a variety of mange species from domestic or wild canids into other naïve wildlife populations can have severe consequences, particularly for small endangered populations (Goltsman *et al.*, 1996). The *Sarcoptes* varieties also cause disease in both domestic and wild species in Namibia namely cattle, pigs, goats, kudu (*Tragelaphus strepsiceros*), hartebeest (*Alcelaphus buselophus*), black-backed jackals (Zumpt & Ledger 1973) and springbok (*Antidorcas marsupialis*) (Schneider 1994). Domestic dogs and jackals may therefore act as a source of re-infection for both wild and domestic species but little is known about the basic pathology of the disease, and the susceptibility and immunity of wild canids.

The *Sarcoptes scabiei* var. *canis* is common in domestic dogs on the Namibian coast and is thought to have been introduced to the coastal jackal population at Cape Cross in the late 1980s by an infected dog originating from one of the nearby coastal towns (pers. comm. Dr. H. Reuter, veterinarian, Ministry of Environment and Tourism). Jackals proved to be highly susceptible and the infection spread very rapidly along the coast, resulting in high morbidity and mortality despite culling efforts by the MET. To date, there are no known published studies of mange in this population although an internal report was compiled for the Ministry of Environment (pers. comm. H. Reuter). The aim of this investigation (Vila Garcia *et al.*, in prep.) is to describe the pathology of the disease in jackals, determine the prevalence of infection and exposure, and to test an ELISA developed for the diagnosis of the disease in dogs (Bornstein & Zakrisson 1994, Lower *et al.*, 2001) as traditional skin scrape and biopsy methods are known to be unreliable (Bornstein & Zakrisson 1994).

CAV-1, also surveyed in this study, causes infectious canine hepatitis and clinical disease has been reported in a range of canid species including dogs, coyotes, foxes and bears (Greene 1998). Transmission between species has been documented (Cabasso 1981) and the high prevalence of naturally occurring VN antibodies in wild and domestic canid populations suggests that non-lethal infection is very common (Holzman *et al.*, 1992, Johnson *et al.*, 1994, Greene 1998). CAV-1 is not thought to be a major threat to the viability of the Ethiopian wolf, as determined by a study of the prevalence of infection and population trends (Laurenson *et al.*, 1998), but infection in bears or foxes has more severe consequences (Cabasso 1981, Pursell *et al.*, 1983).

Jackals may be used as disease sentinels (Alexander & Appel 1994), the small cross-sectional serosurveys providing a 'snapshot' of the diseases to which both domestic dogs and wildlife may be exposed to by jackals. Longitudinal serosurveys provide a better indication of whether a pathogen is endemic or epidemic in the population as an increasing trend in seroprevalence with age in a cross-sectional study may be due to an endemic persistence (or differential rates of exposure or mortality in an epidemic).

### **5.3 Aims**

The main diseases of interest in this study are sarcoptic mange, CPV-2, and CAV-1; CHV and helminth infections are also considered in order to construct a more complete disease profile of the coastal jackal and dog populations. Table 5-1 provides a summary of the main characteristics of CPV-2, sarcoptic mange and CAV-1. The aims of this study may be summarised as follows:

1. To determine whether sarcoptic mange, CPV-2, and CAV-1 are endemic or epidemic in the jackal and dog populations of the coast. This will be achieved by considering the overall seroprevalences, the temporal and age patterns of exposure, the basic reproductive numbers, the available disease data and the pathogens' characteristics.
2. To evaluate an ELISA for the diagnosis of sarcoptic mange infection in wild canids.
3. To determine the prevalence of exposure to CHV in dogs and jackals and the prevalence of helminth infections in jackals.

**Table 5-1: A summary of the main characteristics of sarcoptic mange, CPV-2, and CAV-1.**

Pathogen	Infective period outside host	Transmission	Clinical signs	Mortality
Sarcoptic mange	Several weeks at high relative humidity and low temperature	Direct and indirect contact	Acute signs are a result of a hypersensitivity reaction. Crusted, hyperkeratotic lesions with alopecia and hypermelanosis, emaciation	High in animals of all ages in naïve populations but likely to be lower once endemic
CPV-2	5 months or longer on inanimate objects	Oronasal exposure to faeces or indirect contact	Vomiting (severe), diarrhoea, anorexia, dehydration, cardiac myopathy	High in animals of all ages in naïve populations but only in pups once endemic
CAV-1	Days at room temperature	Oronasal contact with urine, other secretions, and indirect contact; possibly ectoparasites	Coughing, abdominal tenderness, icterus, “blue eye” – corneal opacity; CNS signs including depression, disorientation and seizures	Most commonly seen in dogs less than 1 year of age but may affect susceptible dogs of any age

References: (Greene 1998, Hoskins 1998, Barker & Parrish 2001, Bornstein *et al.*, 2001).

## 5.4 Materials and methods

### 5.4.1 Sarcoptic mange

#### 5.4.1.1 Field examinations and sampling of mange lesions

The distribution of the visible mange lesions was outlined on dorsal and ventral sketches of the jackals as described in Chapter 2 (section 2.5.2); it is possible that very small and/or early stage lesions may have been missed in this examination. The percentage of the total body surface area which was affected was calculated from a fine grid (standard graph paper) superimposed onto each sketch.

Skin scrapes and biopsies from the mange lesions were processed (see Chapter 2, section 2.5.2 for sampling and storage) and analysed by Dr. G. Vila Garcia at the Royal Veterinary College, London (Vila Garcia *et al.*, in prep.). Scrapes were centrifuged and the sediment examined microscopically. Skin biopsies were embedded in paraffin and 4µm sections stained in haematoxylin and eosin for histopathological examination.

#### 5.4.1.2 Sarcoptic mange ELISA

Jackal sera from 2001 and 2002 and dog sera from all three sampling years were tested using a commercial kit (Imovet Sarcoptic, *in vitro* determination of canine sarcoptic-specific IgG, Imovet bg, vetproducts, Switzerland). Aliquots of 1:50 serum dilutions were added to whole body extract of *Sarcoptes* var. *vulpes* in microtitre wells and incubated for 1 hour at 37°C. After washing, a polyclonal goat anti-canine IgG (a conjugate with alkaline phosphatase) was added to each test well and incubated for 30 minutes at 37°C. The plates were washed and substrate (p-nitrophenyl phosphate) added to each well. Following a 45 minute incubation at room temperature, the reaction was stopped with 3M NaOH. The activity of the bound alkaline phosphatase was measured with p-NPP (p-nitrophenyl phosphatase). The absorbance (optical density) of the chromogen was determined at 405nm. The

positive and negative controls were sera of known positive and negative dogs, provided with the kit.

The final result for each sample was calculated as a percentage using Equation 1.0 as described in the test instructions.

$$\frac{(OD \cdot Sample - OD \cdot Negative \cdot Control)}{OD \cdot Positive \cdot Control} \times 100 \quad \text{Equation 1.0}$$

The OD of the negative and positive controls were calculated from the averages of all controls taken from each of three test batches. Negative samples were classified as those with a modified optical density value of <20% compared to the positive control (100%), questionable results were classed as those in the 20-25% range and the sample was considered positive with a percentage >25%. Questionable results were only re-classified as positive if the animal was seen to have lesions in the field examination; questionable results were not modified if the animal was found to be negative from the physical examination.

#### **5.4.2 HAI test for canine parvovirus types 2a and 2b**

Porcine erythrocytes were used for the HAI (Haemagglutination inhibition) test (Churchill 1982). Briefly, two-fold serial dilutions of the jackal and dog sera were incubated with CPV-2 antigen (4HA units) for 1 hour at 37°C in 96-well microtitre plates. An equal volume of 1% solution of porcine erythrocytes was then added to each well and the samples incubated at 4°C for 1 hour. The plates were then read taking the end point titres as the last serum dilution to show any haemagglutination. Reciprocal titres  $\geq 64$  were considered positive based on the frequency distribution of the titres ( $\log_{10}$ ).



### 5.4.3 Virus neutralisation tests

Tests were performed using the methodology described for CDV in Chapter 3. MDCK cells were used for CAV-1 and A72 cells for CHV. For CAV-1, reciprocal titres of  $\geq 32$  were considered positive based on the frequency distribution of the titres ( $\log_{10}$ ). A random subset of jackal and dogs were tested for CHV and in this case only the first two serum dilutions (1:8 and 1:32) were used and a positive sample recorded as one which showed a cytopathic effect in one or more wells of the first serum dilution; titres for CHV were not calculated. For all of these laboratory tests, a number of serum samples had to be excluded due to low volume or cytotoxic effects on cell cultures.

### 5.4.4 Jackal faecal samples

#### 5.4.4.1 Sample collection

Fresh faecal samples were collected from captures and the field *ad hoc* throughout the study period. In addition, a sampling of the Cape Cross area was made over the course of January 2003. Faeces were collected every 2-3 days and only sampled if still moist on the inside and fresh enough to attract flies. In order to avoid re-sampling and to maximise the sample numbers, an area of approximately 1km<sup>2</sup> directly behind the northern end of the seal colony was cleared of old and sampled faeces during sampling. Faecal samples from the field were collected as described for captures in Chapter 2 (section 2.5.2) and stored in 10% buffered formalin. Any worms recovered from post-mortems and faecal samples were also preserved in 10% buffered formalin.

#### 5.4.4.2 Sample analysis

For the identification of parasites and a semi-quantitative analysis of the faecal samples, faecal samples ( $n=35$ ) in 10% buffered formalin and any worms collected from faeces or *post-mortem* were submitted to Drs. M. Fox and C. Pollard at the

Royal Veterinary College (London) for processing and analysis (C. Pollard *et al.*, in prep.). Some samples could not be submitted for examination as the volume of faeces was too small. The methodology for the faecal analysis by Dr. C. Pollard is described in the draft manuscript in the Appendices (section 8.3.1).

## **5.4.5 Data analyses**

### **5.4.5.1 Seroprevalence data**

Logistic regression analyses were performed using seroprevalence data for sarcoptic mange, CPV-2, CAV-1 and CHV (SPLUS 2000, MathSoft Inc.) as described in Chapter 2 (section 2.10). The data for the sarcoptic mange analysis included those individuals whose result was modified from questionable to positive. Seropositivity for each disease was classified as a binary variable (seropositive 1, seronegative 0) and all other variables (species, age class, sex, location, sampling year) were classified as categorical. The age analyses were repeated with and without the domestic dogs from 2003 and since there was no qualitative change in the results, the results of the age analyses without these individuals are presented.

### **5.4.5.2 Sarcoptic mange data**

Logistic regression models were fitted (SPLUS 2000, MathSoft Inc.) to determine if age class or sex, controlling for sex and age class respectively, were significant predictors of the occurrence of mange lesions (classed as 1 or 0). The models were simplified as described for the logistic regression analyses of seropositivity (Chapter 2, section 2.10); the results of the full models are presented in tables. Univariate logistic regression models were fitted to determine if the ELISA result (classed as 1 or 0 for positive and negative respectively and excluding any questionable results) was predictive of the occurrence of visible mange lesions.

Univariate general linear models (SPLUS 2000, MathSoft Inc.) were fitted to test for a linear relationship between age, sex and body weight and the area coverage of

mange lesions. Due to the limited sample size juveniles and sub-adults were classed as one age class (age class 1) and adults as another (2). Sex was also classified as a categorical variable (males '1', females '2'). Bodyweight, measured to one decimal place was treated as a continuous variable. The proportion of the body area affected was transformed using the arcsine transformation (Chapter 2, section 2.9).

A Spearman rank correlation coefficient was calculated to test for an association between the ELISA converted OD value (%) and total area of mange lesions (%) as the data were not normally distributed and could not be satisfactorily transformed for a parametric test.

The probability that an animal which tests positive or negative is truly positive or negative is termed the predictive value of the test (Thrusfield 1995a). The positive and negative predictive values of the ELISA were calculated (Thrusfield 1995a) from the prevalence, specificity and sensitivity of the ELISA (Table 5-2 and Equations 2.0-2.3). Calculations were based upon the detection of mange lesions in field examinations.

**Table 5-2: The calculation of the positive and negative predictive values of the sarcoptic mange ELISA.** Adapted from Thrusfield (1995).

ELISA status	True status (visible mange lesions)		Totals
	Diseased	Not diseased	
Diseased	<i>a</i>	<i>b</i>	<i>a+b</i>
Not diseased	<i>c</i>	<i>d</i>	<i>c+d</i>
Totals	<i>a+c</i>	<i>b+d</i>	<i>a+b+c+d</i>

$$\text{Sensitivity} = a / (a + c)$$

*Equation 2.0*

$$\text{Specificity} = d / (b + d)$$

*Equation 2.1*

$$\text{Positive predictive value} = a / (a + b)$$

*Equation 2.2*

$$\text{Negative predictive value} = d / (c + d)$$

*Equation 2.3*

#### 5.4.5.3 Other analyses

A Fisher's Exact test was performed to test for differences between jackals and dogs in the proportions of individuals seropositive to two or more pathogens.

#### 5.4.5.4 The basic reproductive number, $R_0$

The basic reproductive number ( $R_0$ ), is defined as the number of secondary infections arising from one infected individual introduced into a susceptible population (Anderson & May 1991).  $R_0$  was calculated for CAV-1 in jackals and for CPV-2 and CAV-1 in dogs using Equation 3.0, the steady state fraction of susceptible individuals given by

$$R_0 = 1/x$$

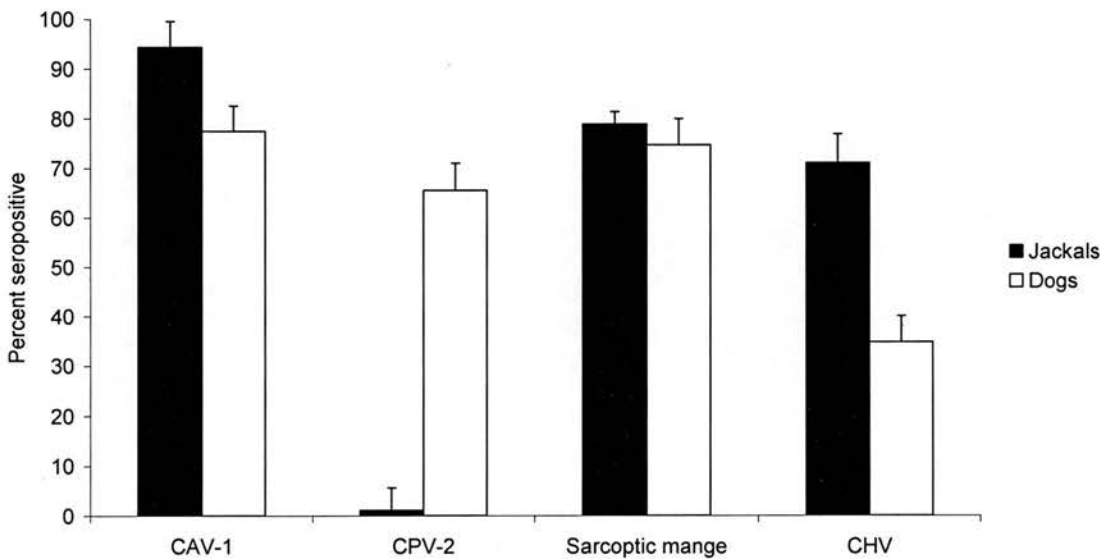
*Equation 3.0*

where  $x$  is the proportion seronegative for a pathogen; this assumes a stable endemic state with weak homogeneous mixing between individuals (Anderson & May 1991). Although suitable for the coastal jackal population these assumptions were likely violated for the coastal dog population as a whole, as there was little evidence of contact between dogs from different towns. But contact between dogs in towns is thought to be more homogeneous (Perry 1993) and so Equation 3.0 was applied to the proportions seronegative (across all sampling years) from Swakopmund and Walvis Bay combined and Lüderitz separately. The 95% CI for the  $R_0$  estimates were calculated from the 95% CI of the proportions seronegative using Equation 3.0.

# 5.5 Results

The results of the serosurveys, physical examinations, post-mortem findings, histopathology and the calculation of  $R_0$ , if performed, are presented separately for sarcoptic mange, CPV-2 and CAV-1. For CHV only the serology results are presented. The seroprevalence of each pathogen is given in Figure 5-1.

**Figure 5-1: The seroprevalence of CAV-1, CPV-2, sarcoptic mange and CHV in jackals and domestic dogs sampled between 2001 and 2003.** Standard errors are shown above the bars. Sample sizes jackals;domestic dogs: CAV-1 88;80, CPV-2 90;90, Sarcoptic mange 71;91, CVH 45;43.



## 5.5.1 Sarcoptic mange

### 5.5.1.1 Serosurvey

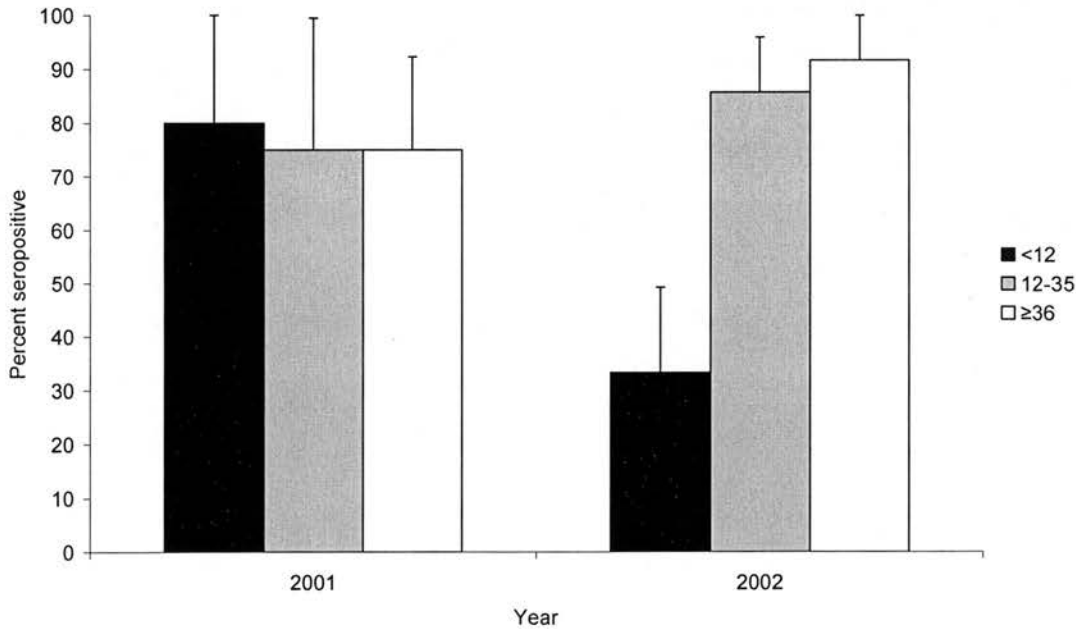
The seroprevalence of sarcoptic mange in jackals (78.9%,  $n=71$ , 4 questionable results, 95% CI 67.6-87.7) and dogs (74.7%,  $n=91$ , 1 questionable result, 95% CI 64.5-83.3) did not differ significantly (full model:  $\chi^2_1 = 2.53$ ,  $P = 0.112$ ).

**Table 5-3: Logistic regression models of mange seropositivity in jackals.**

Term	$\chi^2$	<i>df</i>	Residual <i>df</i>	<i>P</i> -value
Age class	7.70	2	63	0.021
	Coefficient value	s.e.		
Age class 2	1.50	0.78		
Age class 3	2.08	0.82		

The age of a jackal was the only significant predictor of mange seroprevalence (Table 5-3) with the frequency of exposure increasing with age. But as can be seen from Figure 5-2, this was only significant in 2002 ( $\chi^2_2=13.54$ ,  $P=0.001$ ) and not in 2001 ( $\chi^2_2=0.35$ ,  $P=0.838$ ). Overall, exposure did not vary significantly between years and was high in both 2001 and 2002 with seroprevalences of 76.5% (95% CI 50.1-93.2) and 79.6% (95% CI 66.5-89.4) respectively.

**Figure 5-2: The age seroprevalence of jackals sampled in 2001 and 2002 to sarcoptic mange.** Standard errors are shown above the bars. Age classes in months.  $n=71$ .



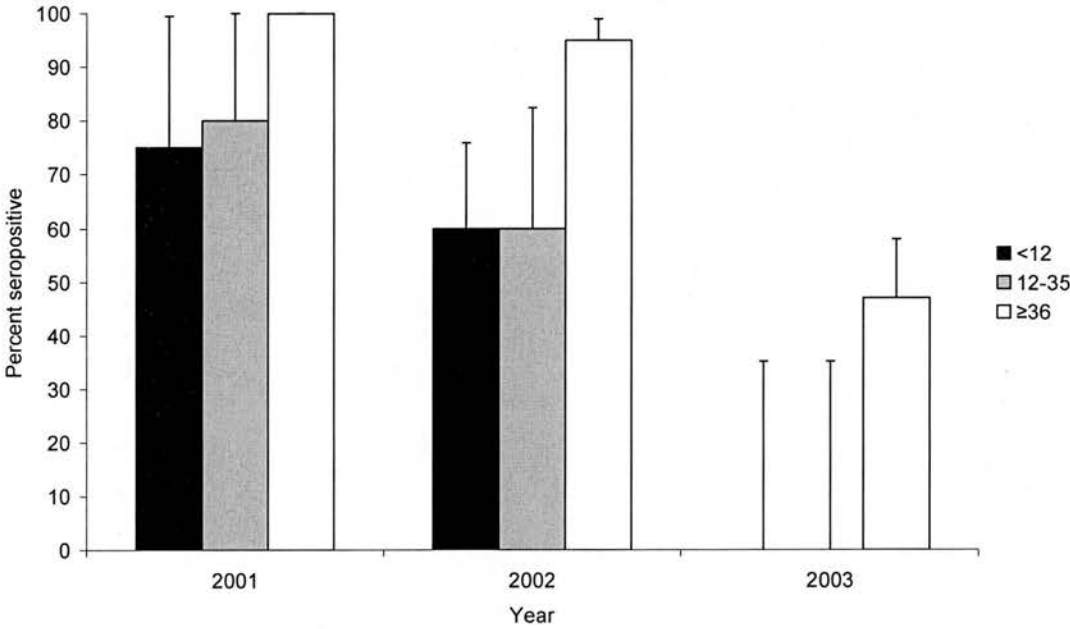
In dogs, a number of factors were predictive of exposure to sarcoptic mange (Table 5-4). Taking into account the effect of location, the frequency of seropositives decreased over the course of 2001-3. Accounting for the effects of year and age, there was a lower frequency of seropositives in Lüderitz compared to Walvis Bay and Swakopmund combined. Controlling for location, older dogs were more likely to

be seropositive than younger dogs (Figure 5-3) but this effect was only significant for 2002 ( $\chi^2=9.11$ ,  $P=0.011$ ) but not 2001 ( $\chi^2=2.49$ ,  $P=0.288$ ).

**Table 5-4: Logistic regression models of mange seropositivity in dogs.**

Term	$\chi^2$	<i>df</i>	Residual <i>df</i>	<i>P</i> -value
Year	15.44	2	69	<0.001
Location	8.74	1	69	0.003
Age class	12.76	2	52	0.002
	Coefficient value	s.e.		
Year 2	-1.56	0.89		
Year 3	-1.99	0.84		
Location 2	-3.16	1.35		
Age class 2	0.57	0.97		
Age class 3	2.30	1.19		

**Figure 5-3: The age seroprevalence of dogs sampled between 2001 and 2003 to sarcoptic mange.** Standard errors are shown above the bars. Age classes in months.





### 5.5.1.2 The occurrence of mange lesions

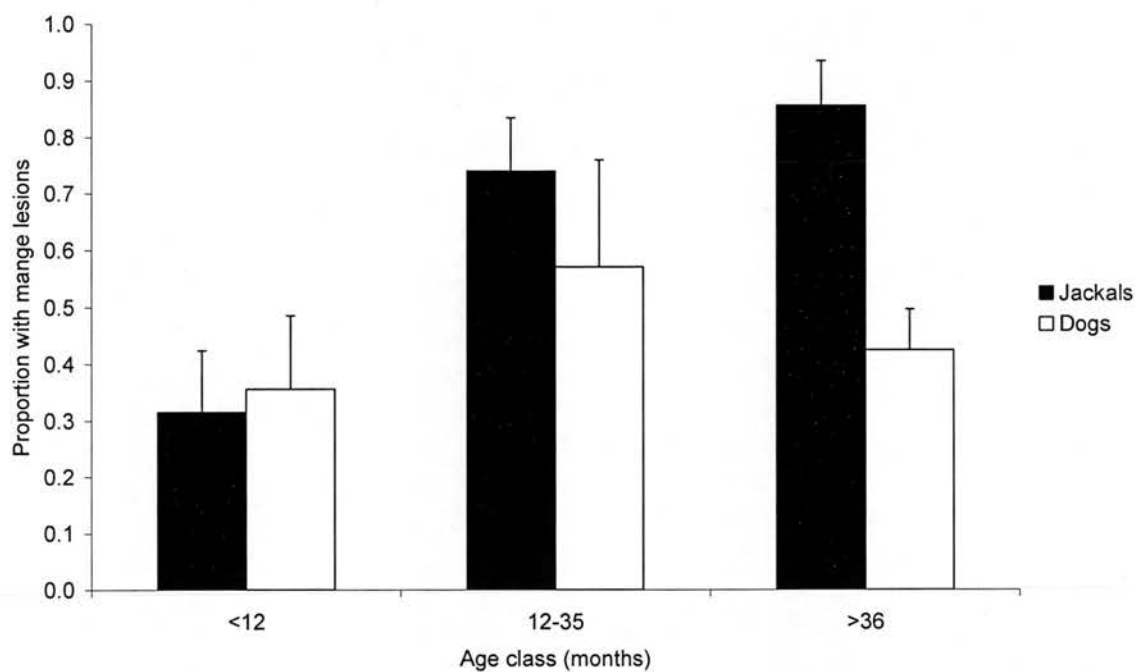
A summary of the prevalence of mange lesions in jackals and dogs is given in Table 5-5. Of 81 jackals for which mange status was recorded over the course of September 2001 to February 2003, lesions were present in 69.1% (95% CI 57.9-78.9) of individuals. This is higher than the prevalence of lesions observed in domestic dogs over the same period (44.6%,  $n=83$ , 95% CI 33.6-55.9). The prevalence of mange lesions in jackals was very similar in 2001 and 2002.

**Table 5-5: The prevalence of observed mange lesions in jackals and dogs, 2001-2003.**

Species	Year	Prevalence % ( $n$ )	95% CI
Jackals	All years	69.1 (81)	57.9-78.9
	2001	64.7 (17)	38.3-85.8
	2002	68.9 (61)	55.7-80.1
	2003	100.0 (3)	29.2-100.0
Dogs	All years	44.6 (83)	33.6-55.9
	2001	93.3 (15)	68.1-99.8
	2002	52.8 (36)	35.5-69.6
	2003	12.5 (32)	3.5-29.0

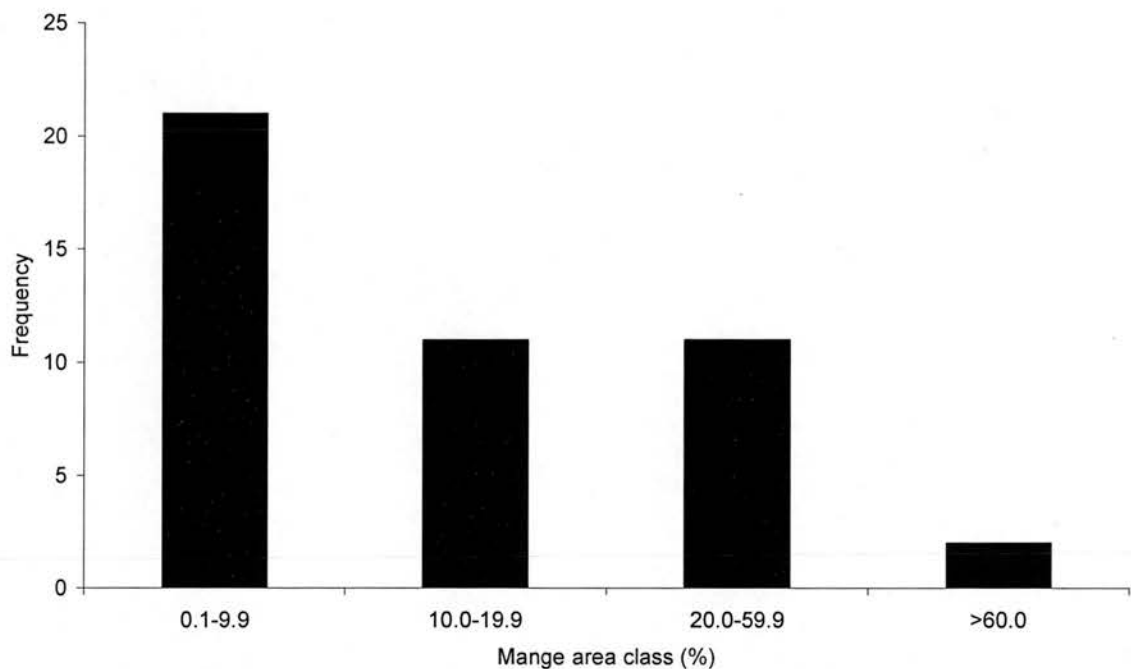
Controlling for age class, male and female jackals did not differ in the numbers with mange lesions ( $\chi^2_1=0.04$ ,  $P=0.834$ ) and the number of jackals with mange lesions increased with age class ( $\chi^2_2=16.63$ ,  $P<0.001$ , Figure 5-4). In dogs neither age class (Figure 5-4) or sex were predictive of the occurrence of mange lesions (age class:  $\chi^2_2=0.59$ ,  $P=0.744$ , sex:  $\chi^2_2=0.002$ ,  $P=0.959$ ).

**Figure 5-4: The proportions of dogs and jackals in each age class with mange lesions.** Standard errors are shown above the bars. Jackals  $n=81$ , domestic dogs  $n=68$ .



As can be seen from Figure 5-5, the majority of individuals had small areas of mange typically on the elbows, tail or peri-anal area (range percentage coverage $\pm$ SD: 0.3-65 $\pm$ 15.2). Two individuals had very severe and debilitating infections covering over 60% of the body area.

**Figure 5-5: The frequency of jackal mange lesion areas in 4 different area classes.**  
*n*=45.



Mange area was not predicted by the age class ( $\chi^2_1=540.2$ ,  $P=0.0617$ ), sex ( $\chi^2_1=152.4$ ,  $P=0.328$ ) or bodyweight of jackals ( $\chi^2_1=139.8$ ,  $P=0.349$ ).

Several cases in jackals were observed over the course of the field periods at Cape Cross in 2002 which suggested that the appearance of mange lesions in some individuals may be linked to the winter months of low food availability (August to October) and that recovery from infection is possible; recovery may be associated with the abundance of food after the birth of the seal pups in November. Three cases are described below.

On the 8<sup>th</sup> of July a sub-adult male was captured which had no visible signs of mange infection and was in good condition with a score of 3; at this time the animal was clearly seropositive with an OD result of 64.5%. By the 31<sup>st</sup> of August the same individual had developed a typical ‘rat tail’ which was completely devoid of hair and darkened in colour; the hind legs were also affected below the hock. On the 13<sup>th</sup> of October the fur on the tail showed signs of re-growth at the base and this had progressed by the time the animal was sighted again on the 19<sup>th</sup> of November. On the 23<sup>rd</sup> of December there were no areas of alopecia and although the tail was not as thick with hair as unaffected individuals it was greatly recovered.

A juvenile female captured on the 16<sup>th</sup> of July at Cape Cross was in poor condition with a score of 1 but had no visible mange lesions and tested seronegative. A sighting on the 30<sup>th</sup> of October showed small mange lesions characterised by alopecia and darkening of the skin on the lower half of the hind legs and thinning of the fur at the base and end of the tail.

A sub-adult female captured on the 28<sup>th</sup> of October had small circular lesions scattered along the length of the legs but was in very good condition with a score of 5; this individual tested seropositive with an OD result of 74.8%. The same individual was sighted on the 22<sup>nd</sup> of November when the mange lesions had spread to affect the whole of the head, legs and tail.

#### 5.5.1.3 Histopathology

The histopathological changes (Table 5-6) associated with mange infection in jackals included epidermal hyperplasia, parakeratosis, hyperkeratosis, hypergranulosis, spongiosis, hypermelanosis, oedema, dermatitis and dermal oedema. Hyperplasia was frequently associated with hypermelanosis, the formation of rete ridges and spongiosis. The perivascular dermatitis varied greatly in severity from a mild to marked inflammatory response. Active infections i.e. the presence of *Sarcoptes scabiei* mites in scrapes or biopsies, were only detected in four individuals.

**Table 5-6: A summary of the histopathological changes observed in the skin biopsies taken from mange lesions of jackals.** The frequency of the different histopathological changes is listed for each of the major layers of the skin;  $n=39$ , including unconfirmed cases.

Skin structure	Pathology	<i>n</i>
Epidermis	Hyperplasia	34
	Sub-corneal mites	4
	Stratum corneum	Parakeratosis
		Hyperkeratosis
		Eosinophilic micropustules
	Stratum granulosum	Serocellular crust
		Hypogranulosis
		Hypergranulosis
	Stratum spinosum	Spongiosis
		Hypermelanosis
		Inflammatory Infiltrate
Dermis	Congestion	2
	Fibrosis	8
	Superficial	Oedema
		Dermatitis
		Inflammatory Infiltrate
		Fibrosis
	Deep	Inflammatory infiltrate
		Orphaned sebaceous glands
		Oedema
		Eosinophilic infiltration
Hypodermis		2

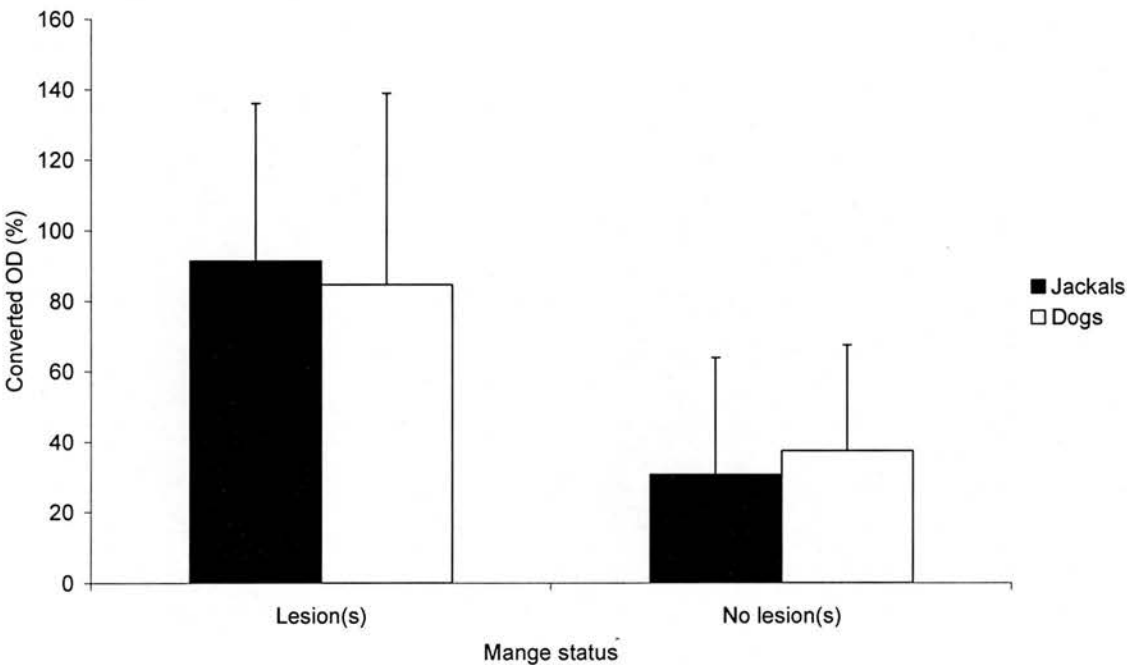
#### 5.5.1.4 Evaluation of the ELISA

For the jackals with and without lesions, the mean converted OD readings were 91.4% (range±SD: 8.5-192.8 ± 44.70%) and 30.7% (range±SD: 2.3-124.9±33.25%) respectively (Figure 5-6). The mean converted OD readings for jackals with mites observed in the skin scrape and/or biopsy ( $n = 9$ ) was 124.3% (range±SD: 224.-192.8 ± 58.12%). In dogs the mean converted OD readings and ranges for those with and without lesions were 84.7% (range±SD: 9.6-198.5 ± 54.36%) and 37.5% (range±SD: 0.2-118.9±30.06%) respectively.

The ELISA result was a significant predictor of the occurrence of mange lesions for both jackals ( $\chi^2=26.93$ ,  $P<0.0001$ ) and dogs ( $\chi^2=12.84$ ,  $P<0.001$ ). As can be seen from the Figure 5-6 there was no significant difference between converted OD values

between species in the groups with and without lesions respectively (Wilcoxon Rank Sum Test:  $Z = -0.87$ ,  $P = 0.386$  and  $Z=1.29$ ,  $P = 0.196$ ). The converted OD reading of the ELISA was highly predictive of the area of the mange lesion ( $\rho = 0.439$  and  $P = 0.007$ ).

**Figure 5-6: The mean converted ELISA OD values for jackals and dogs with and without visible mange lesions.** Standard deviations are shown above the bars. Jackals  $n=69$ , domestic dogs  $n=76$ .



The positive and negative predictive values for jackals and dogs were calculated as shown in Tables 5-7 and 5-8 respectively.

**Table 5-7: The calculation of the positive and negative predictive values of the ELISA for jackals.**

ELISA Status	True status (visible mange lesions)		Totals
	Diseased	Not diseased	
Diseased	47	7	54
Not diseased	2	11	13
Totals	49	18	67
Sensitivity	95.9%		
Specificity	61.1%		
	95% CI of predictive values		
Positive predictive value	0.870	0.751-0.946	
Negative predictive value	0.846	0.546-0.981	

**Table 5-8: The calculation of the positive and negative predictive values of the ELISA for dogs.**

ELISA Status	True status (visible mange lesions)		Totals
	Diseased	Not diseased	
Diseased	34	23	57
Not diseased	3	17	20
Totals	37	40	83
Sensitivity	91.9%		
Specificity	42.5%		
	95% CI of predictive values		
Positive predictive value	0.596	0.458-0.724	
Negative predictive value	0.850	0.621-0.968	

A summary of the results of the sarcoptic mange study in jackals is provided in Table 5-9. Only one individual which had mange lesions resulted seronegative but the skin scrapes and biopsies showed little agreement and only detected mites in less than 15% of cases.



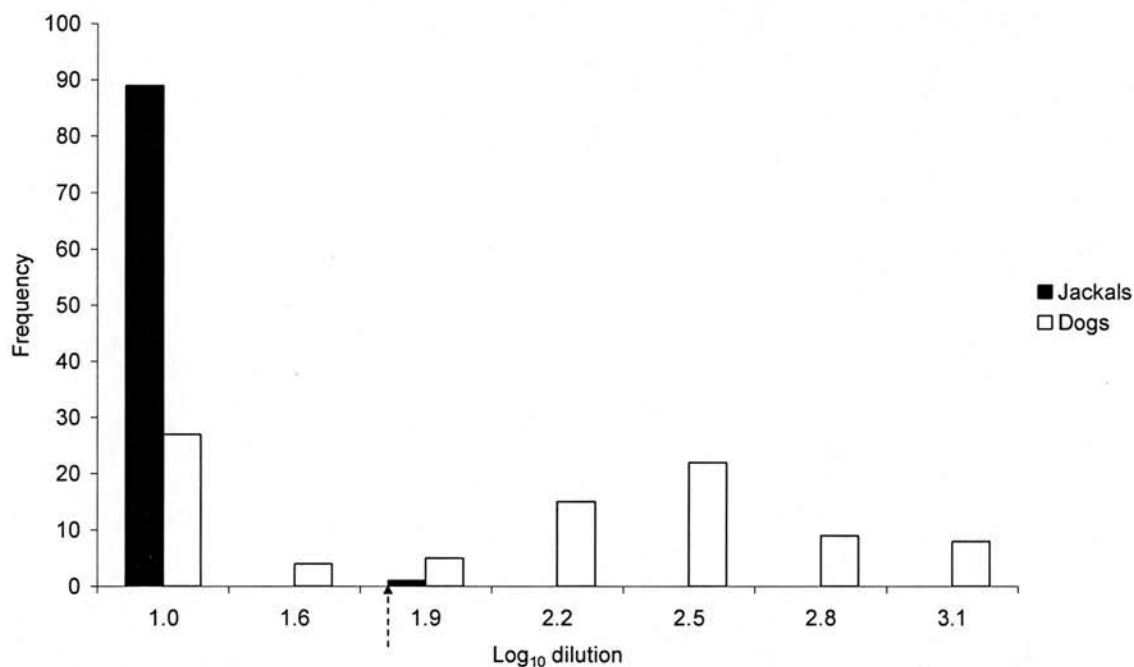
**Table 5-9: A summary of sarcoptic mange in jackals.**

Criteria or diagnostic for mange infection	Numbers of jackals
Skin lesions in those examined	56 of 81
Jackals seropositive	54 of 67
Number with questionable results	2
Number of questionable results modified	2
With lesions and negative by ELISA	1
With mites in skin scrape	6 of 41
With mites in skin biopsy	4 of 39
With mites in scrape or biopsy and negative by ELISA	0
Positive by scrape and negative by biopsy	5
Positive by biopsy and negative by scrape	3
Positive by biopsy and scrape	1
ELISA sensitivity	95.9%
ELISA specificity	61.1%

### **5.5.2 Canine parvovirus type 2**

A more stringent cut-off point of a reciprocal titre of  $\geq 64$  ( $\log_{10}$  1.8, indicated by the arrow in Figure 5-7), rather than the standard cut-off of  $\geq 10$  (1.0), was selected for dogs and jackals, according to the titre frequency distribution. There was no qualitative change in the results of the logistic regression analyses when comparing the same analyses using the two different cut-offs for jackals and dogs.

**Figure 5-7: The frequency distribution of CPV-2 neutralising antibody titres ( $\log_{10}$ ) in jackals and dogs.** The arrow indicates the cut-off point of 1.8 ( $\geq 64$ ). Jackals  $n=90$ , domestic dogs  $n=90$ .

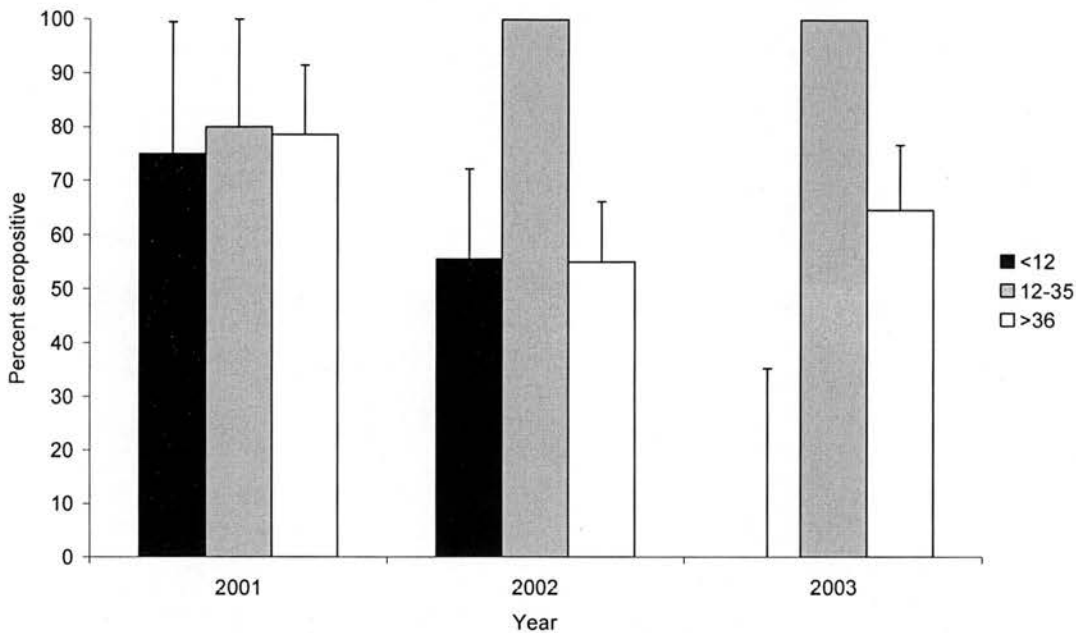


### 5.5.2.1 Serosurvey

Controlling for the effects of year and location, there was a highly significant difference (full model:  $\chi^2_1 = 91.52$ ,  $P < 0.0001$ ) in seroprevalence between jackals (1.1%,  $n=90$ , 95% CI 0.0-6.0) and dogs (65.6%,  $n=90$ , 95% CI 54.8-75.3). The only seropositive jackal was an adult male captured at Cape Cross in 2003.

None of the variables in the logistic regression analyses were significant predictors of CPV-2 exposure in either jackals or dogs (smallest  $P$ -values and largest  $\chi^2$  values for jackals and dogs respectively:  $P=0.193$ ,  $\chi^2=2.34$ ;  $P=0.052$ ,  $\chi^2=3.77$ ). In dogs, the full model for the location term approached significance ( $\chi^2_1=3.77$ ,  $P=0.052$ ). As can be seen from Figure 5-8 there was little variation in seroprevalence between age classes in 2001 whilst 2002 and 2003, the years of the CD epidemic, show markedly different patterns.

**Figure 5-8: The age seroprevalence of dogs sampled between 2001 and 2003 to CPV-2.** Standard errors are shown above the bars. Age classes are in months.  $n=76$ .



**5.5.2.2 The occurrence of disease**

Gross pathological changes consistent with, but not attributable to parvovirus infection, were observed in a number of jackals. An adult female euthanased due to respiratory signs had areas of severe congestion and generalised petechiae in the mucosa of the small intestine; a sub-adult male showed haemorrhagic ulcers and caseous foci in the small intestine; a sub-adult female, found dead, was seen to have bled out from the anus. Swollen and congested intestines were observed in a juvenile male. CPV-2 was not cited as the aetiological agent in any of the histopathological examinations ( $n=14$ ) and only one domestic dog ( $n=6$ ) showed inflammation of the small intestine.

5.5.2.3 The basic reproductive number,  $R_0$

Using Equation 3.0 and the proportions of seronegative dogs from Lüderitz and from Swakopmund and Walvis Bay combined, the  $R_0$  values for CPV-2 in dogs were calculated to be 2.5 and 4.5 for these locations respectively (Table 5-10).

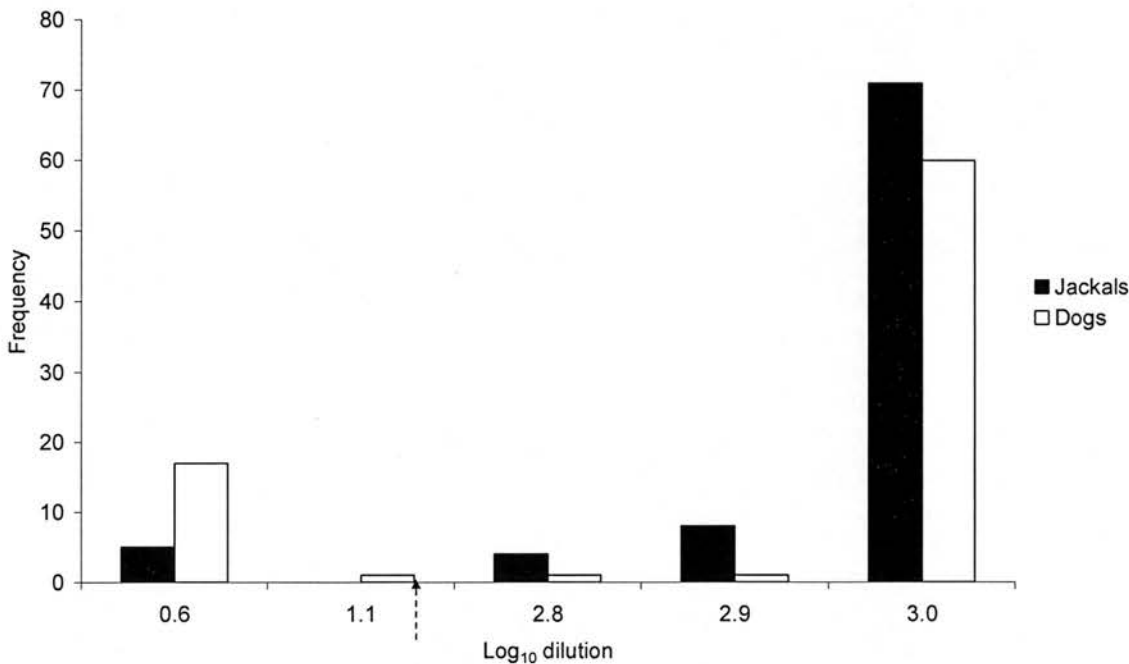
**Table 5-10: The  $R_0$  estimates for CPV-2 in dogs by location.**

Location	Proportion seronegative ( <i>n</i> )	95% CI	$R_0$ estimate	95% CI of $R_0$ estimate
Lüderitz	0.40 (63)	0.28-0.53	2.5	1.9-3.6
Swakopmund & Walvis Bay	0.22 (27)	0.09-0.42	4.5	2.4-11.6

5.5.3 Canine adenovirus type 1

Based on the frequency distribution of titres (Figure 5-9), a cut-off point of a reciprocal titre of  $\geq 32$  ( $\log_{10}$  1.5, indicated by the arrow in Figure 5-9), was selected for dogs and jackals, rather than the standard cut-off of  $\geq 16$  (1.2). There was no qualitative change in the results of the logistic regression analyses when comparing the same analyses using the two different cut-offs for jackals and dogs.

**Figure 5-9: The frequency distribution of CAV-1 VN antibody titres (log<sub>10</sub>) in jackals and dogs.** The arrow indicates the cut-off point of 1.5 (≥32). Jackals *n*=88, domestic dogs *n*=80.



**5.5.3.1 Serosurvey**

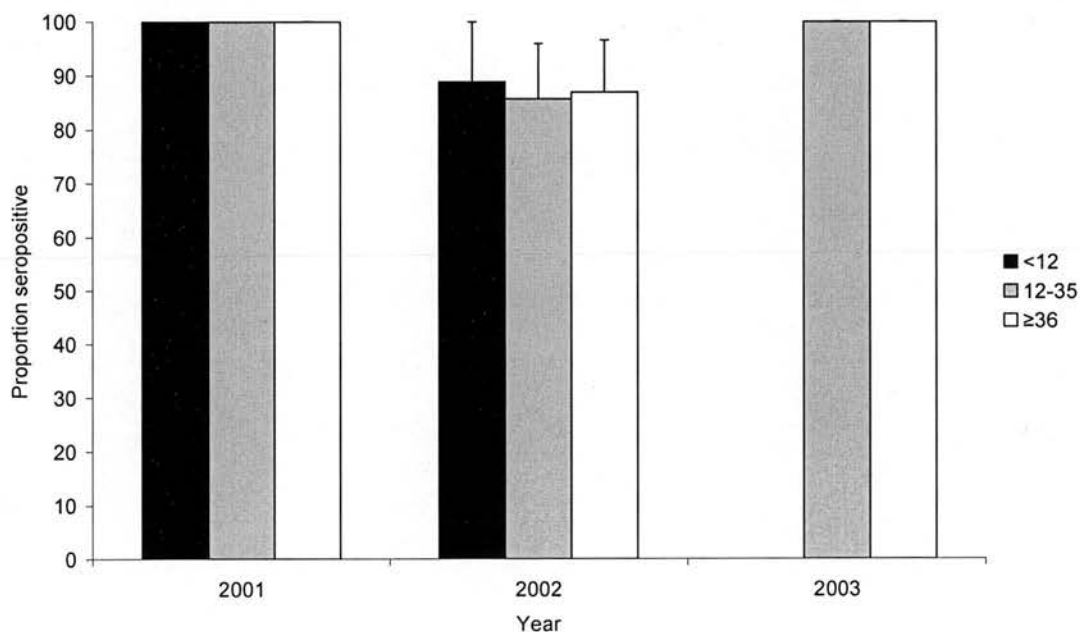
Jackals had a significantly higher seroprevalence (94.3%, *n*=88, 95% CI 87.2-98.1) than domestic dogs (77.5%, *n*=80, 95% CI 66.8-86.1) (full model:  $\chi^2_1=6.92$ , *P*=0.009). Dogs in the younger age classes had a significantly lower chance of being exposed relative to juvenile and sub-adult jackals (interaction age:species:  $\chi^2_1= 6.41$ , *P*=0.041).

For jackals, age class was not a significant predictor of exposure to CAV-1 and exposure was high in all three sampling years (Figure 5-10). Aside from location, no other main effects were significant. Jackals in the Diamond Area were less likely to be seropositive than those in Cape Cross (Table 5-11).

**Table 5-11: Logistic regression model of CAV-1 seropositivity in jackals.**

Term	$\chi^2$	<i>df</i>	Residual <i>df</i>	<i>P</i> -value
Location	5.68	1	80	0.017
	Coefficient value	s.e.		
Location 2	-2.28	0.96		

**Figure 5-10: The age seroprevalence of jackals sampled between 2001 and 2003 to CAV-1.** Standard errors are shown above the bars. Age classes in months. *n*=87.

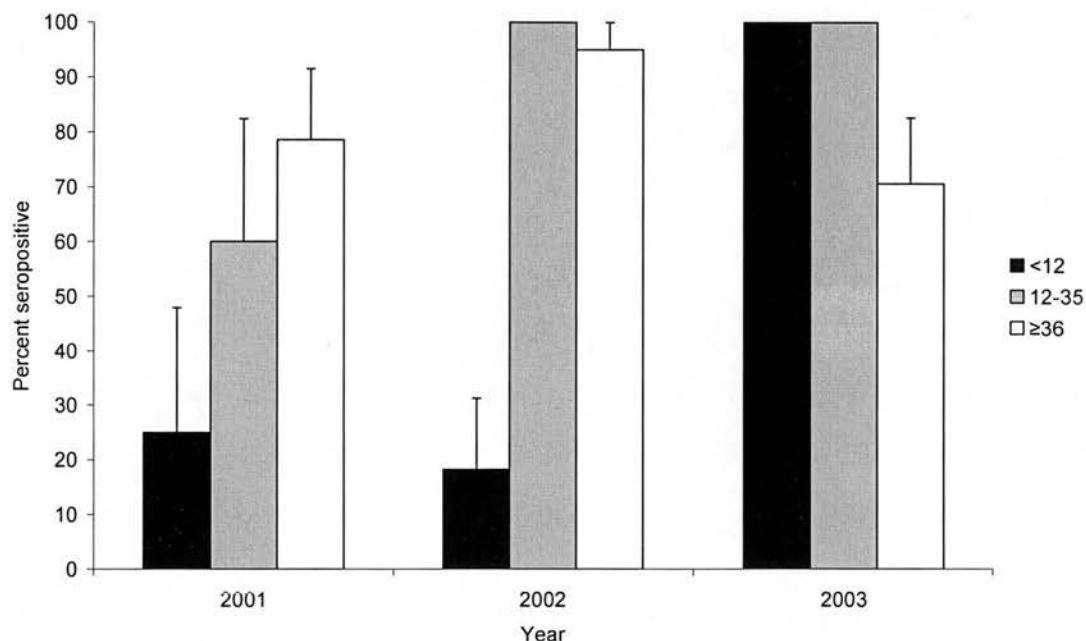


Controlling for sex in domestic dogs, the effect of age class was significant (Table 5-12) with seroprevalence increasing with age (Figure 5-11) and, controlling for age class, males were more likely to be seropositive than females (Table 5-12).

**Table 5-12: Logistic regression model of CAV-1 seropositivity in dogs.**

Term	$\chi^2$	<i>df</i>	Residual <i>df</i>	<i>P</i> -value
Age class	15.02	2	47	0.001
Sex	5.08	1	64	0.024
	Coefficient value	s.e.		
Age class 2	1.74	1.06		
Age class 3	2.52	0.79		
Sex 2	-1.53	0.71		

**Figure 5-11: The age seroprevalence of dogs sampled between 2001 and 2003 to CAV-1.** Standard errors are shown above the bars. Age classes in months.  $n=78$ .



### 5.5.3.2 The occurrence of disease

A number of jackals showed pathological changes of the liver and kidneys consistent with infection with CAV-1 in dogs (Greene 1998). Post-mortem observations from seven individuals included pale, friable, and congested livers with mosaic patterns of necrosis and in one case white foci of necrosis on the surface. The changes in the kidneys included adherence of the epithelial capsule, white striations in the medulla and in one case, multiple white foci of necrosis.

Histopathological changes to the liver included congestion, fatty degeneration, neutrophil infiltration, multifocal lytic necrosis, hepatitis, and extra-medullary haematopoiesis. An adult male was diagnosed with focal hepatitis and hepatitis of unknown aetiology. This was characterised by isolated focal hepatocellular necrosis with isolated foci of inflammatory cells (mainly plasma cells), dilated sinuses and cholestasis. The changes observed in the kidneys included fatty degeneration of the tubules, congestion, nephrosis, and vacuolar changes in the cytoplasm of tubular epithelial cells of the *zona intermedia*. A sub-adult female was diagnosed with chronic segmental nephrosis with fibrosis and a slight inflammatory infiltration.



Non-suppurative nephritis was diagnosed in an adult female showing large areas of lymphoplasmacytic cellular infiltrates, fatty change of the tubules and undefined masses in the tubular cytoplasm.

In dogs, histopathological changes in the liver included congestion with haemorrhages, fatty change in the hepatocytes and hepatocyte degeneration. A three month-old female was diagnosed with focal hepatic necrosis characterised by numerous foci of necrosis, mononuclear infiltrates and centrilobular hepatocytes with vacuolated cytoplasm.

#### 5.5.3.3 The basic reproductive number, $R_0$

Using Equation 3.0 and the proportions of seronegative jackals and dogs (from Lüderitz and from Swakopmund and Walvis Bay combined), the  $R_0$  values for CAV-1 in jackals and dogs were calculated to be 17.6 and, 2.5 and 4.5 respectively (Table 5-13).

**Table 5-13: The  $R_0$  estimates for CAV-1 in jackals and dogs by location.**

Species	Location	Proportion seronegative ( $n$ )	95% CI	$R_0$ estimate	95% CI of $R_0$ estimate
Jackals	All locations	0.06 (88)	0.02-0.13	17.6	7.8-53.5
Dogs	Lüderitz	0.40 (63)	0.28-0.53	2.5	1.9-3.6
	Swakopmund & Walvis Bay	0.22 (27)	0.09-0.42	4.5	2.4-11.6

## 5.5.4 Additional pathogens

### 5.5.4.1 *Canine herpesvirus*

A random subset of jackal ( $n=45$ ) and dog ( $n=44$ , 1 sample was cytotoxic) sera were tested for VN antibodies to CHV. There was a significant difference ( $\chi^2=13.81$ ,  $P<0.001$ ) in seroprevalence between jackals (71.1%, 95% CI 55.7-83.6) and dogs (34.1%, 95% CI 20.5-49.9). A logistic regression analysis revealed a significant decrease in seroprevalence with age class (Table 5-14) in jackals but none of the other factors resulted significant for either species.

**Table 5-14: Logistic regression model of CHV seropositivity in jackals.**

Term	$\chi^2$	<i>df</i>	Residual <i>df</i>	<i>P</i> -value
Age	11.63	2	39	0.003
	Coefficient value	s.e.		
Location 2	-6.41	21.17		

### 5.5.4.2 *Helminth infections*

#### *Post-mortem and histopathological observations*

Worms were recovered from 3 individuals *post mortem* and from 3 faecal samples collected in the field. All 3 *post mortems* revealed severe infections with over 25 hookworms in the large intestine of an adult female which died of unknown causes, adult tape worms in a sub-adult male, and numerous cestodes in the small intestine of an adult male. An unidentified parasitic cyst was found in a section of liver of an adult male (CVL, Windhoek).

The results of the faecal analysis by Dr. C. Pollard are presented in the Appendices (section 8.3.1) as part of a manuscript for publication. The overall prevalence of helminth eggs and protozoan oocysts was high in this jackal population (74.1%,  $n=35$ , 95% CI 53.7-85.4). Nematode, Taenid, and Acanthocephalan eggs were detected as well as Coccidian oocysts. Further details are given in the manuscript.

#### 5.5.4.3 Other infections

An adult female jackal which died of unknown causes had numerous cauliflower-like, firm white growths on the tongue, on the insides of the lips and cheeks and on the palate. Sections in 10% buffered formalin were submitted to Dr. F. Mettler at the CVL (Windhoek) for examination. The infection was diagnosed as oral papillomatosis.

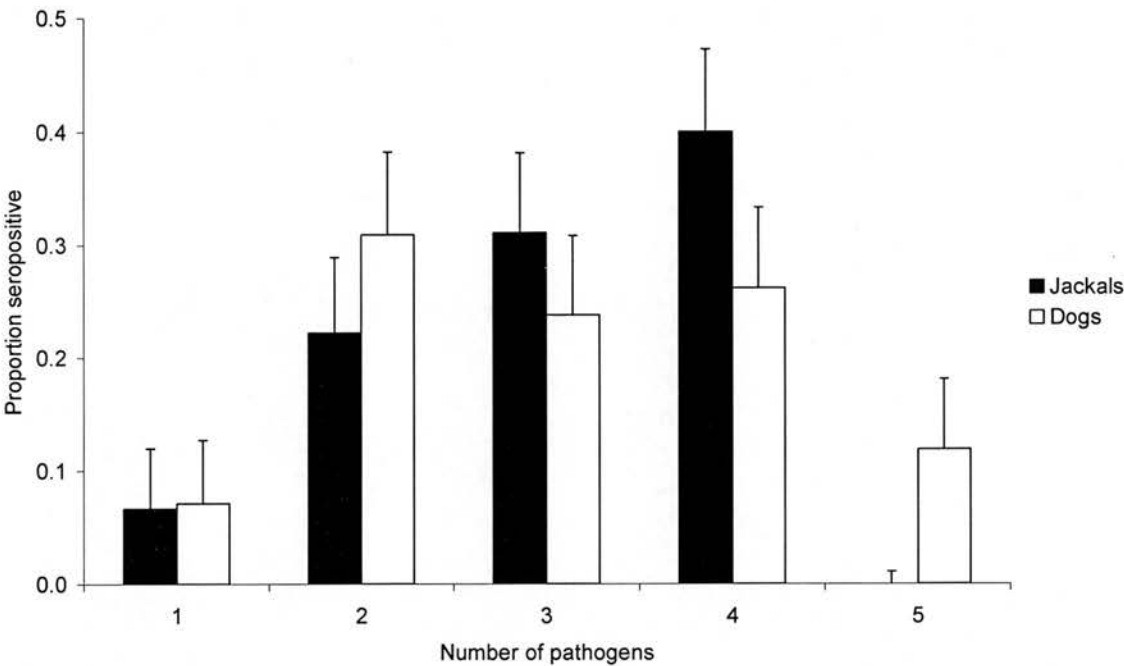
Two blood slides ( $n=32$ ), one from a dog of unknown age and one from an adult female, both sampled in Lüderitz in 2003, contained between one and 3 microfilariae of *Dirofilaria immitis* each; heartworm is a notifiable disease in Namibia. The blood slide from the female also contained leukocytes with morulae of the rickettsial parasite *Ehrlichia canis*.

#### 5.5.5 Exposure to multiple pathogens

Very similar percentages of jackals (93.3%,  $n=45$ , 95% CI 81.7-98.6) and dogs (92.9%,  $n=42$ , 95% CI 80.5-98.5) tested for all 5 pathogens (CDV, CAV-1, CPV-2, CHV and sarcoptic mange) were seropositive for 2 or more pathogens. Although higher proportions of jackals were seropositive to 3 or 4 pathogens (Figure 5-12) there was no difference between jackals and dogs in the number of pathogens individuals were seropositive to (Fisher's Exact Test:  $P = 0.105$ ).

Of the 2 brown hyenas tested, one was seropositive only for CAV-1 but the second was seropositive to CDV, CPV-2 and CAV-1.

**Figure 5-12: The proportions of dogs and jackals seropositive for one or more of CDV, CPV-2, CAV-1, CHV and sarcoptic mange. Standard errors are shown above the bars. Sample sizes as for figure 5-1.**



## 5.6 Discussion and conclusions

### 5.6.1.1 *Sarcoptic mange*

Several factors indicate that sarcoptic mange is likely to be endemic in jackals. Firstly, the disease has persisted since its introduction in the 1980s, as indicated by discussions with Ministry of Environment staff, including the veterinarian who investigated the disease (Dr. H. Reuter). Secondly, the prevalence of exposure and infection were high and stable across sampling years. The low temperatures and high humidity (Scott *et al.*, 2001) of the coastal environment would prolong the survival of mites thus augmenting the prevalence of infection and increasing the chances of (re-)infection (via indirect transmission).

This study provides evidence that the lesions associated with *Sarcoptes scabiei* in jackals show similar histopathological features and a similar lesion distribution on the body, as dogs, coyotes and foxes (Pence *et al.*, 1983, Morner & Christensson 1984, Scott *et al.*, 2001) and that jackals, like foxes (Little *et al.*, 1998a), are capable of recovering from infection, the observations of recovery in this study supporting the earlier findings of Dr. H. Reuter (unpublished report, Ministry of Environment and Tourism).

The knowledge of immunity to mange is limited, but dogs and experimentally infected red foxes are not resistant to re-infection (Little *et al.*, 1998a, Bornstein *et al.*, 2001) and the same may be true of jackals. Cellular immunity is thought to be more important in providing protection than humoral immunity (Arlian *et al.*, 1994) which is known to be of short duration in dogs (Arlian *et al.*, 1994, Bornstein & Zakrisson 1994, Arlian *et al.*, 1996b, Lower *et al.*, 2001). Therefore the increasing age-seroprevalence trends seen in jackals and dogs may be due to the removal of infectious individuals by CDV in 2002, which delayed exposure in the younger age classes, rather than an increasing probability of exposure with age. In the absence of CDV, the force of infection may be high enough that jackals are exposed at a very young age and the high density in this population is likely to ensure re-infection or re-exposure if immunity wanes. The notion of seasonal changes in the prevalence

and intensity of infection remains to be further explored but a decrease in food availability, occurring when most of the seals are out to sea prior to the breeding season in September and October, may well result in reduced resistance to infection.

A number of factors, other than age class, were significant predictors of seropositivity in dogs. The trend of decreasing seroprevalence across sampling years may be due to the removal of infected individuals due to CDV in 2002 and 2003. CDV is known to be immunosuppressive (Greene & Appel 1998) which may result in the mortality of individuals with mange infections, particularly those with higher mite burdens whose immune systems cannot control the infection. This immunosuppressive effect may also occur in jackals but due to the small sample sizes it was not possible to determine if jackals with larger mange lesion areas were more likely to die during the CD epidemic. The differences in seroprevalence between locations may be due to the smaller population size in Lüderitz.

The collective opinion of Ministry of Environment staff working in the coastal area was that the jackal population has not recovered to the level prior to the introduction of sarcoptic mange. Mange epidemics in coyote and red fox populations resulted in population declines of both species (Lindstorm & Morner 1985, Pence & Windberg 1994). But mange has had little long-term effect on the coyote population and mortality is now thought to be compensatory (Pence & Windberg 1994) in this species and the fox population did show signs of recovery (Lindstorm *et al.*, 1994). It is possible that the jackal population is now recovering from the introduction of mange by the breeding of mange-resistant individuals.

The Imovet ELISA was highly effective at predicting the occurrence of mange lesions in both jackals and dogs, as indicated by the logistic regression analysis and the sensitivity values for both species. The sensitivity values for jackals and dogs in this study are comparable to those of other studies in dogs with sensitivity of 84.2% and 83% (Curtis 2001, Lower *et al.*, 2001). The sensitivity values in jackals and dogs were due to 2 and 3 individuals respectively which did not have demonstrable antibodies despite the presence of mange lesions. These false negative results may be

due to the delay between infection and seroconversion as dogs only seroconvert 3 to 5 weeks post exposure or 1 to 3 weeks after the onset of clinical signs, (Bornstein & Zakrisson 1994)). Alternatively these animals may have been recovering as measurable antibodies were only found between 1-4.5 months post treatment in dogs (Lower *et al.*, 2001), or, the antibodies may have decayed due to the freeze-thaw cycles of the samples.

The specificity in dogs and jackals of this study are much lower than those of the other ELISA evaluations (Bornstein *et al.*, 1996, Curtis 2001, Lower *et al.*, 2001). The low specificity values were due to 7 jackals and 23 dogs which had demonstrable antibodies but not mange lesions. These low values may be explained by the use of mange lesions as a tool to diagnose infection rather than the microscopic identification of mites and their ova, or the presence of clinical signs and response to treatment (Bornstein *et al.*, 1996, Curtis 2001, Lower *et al.*, 2001). The smaller lesions would be more likely to be missed during the examinations, particularly in dogs, as the removal of carcasses by the Town Council authorities limited the examination time.

The use of *Sarcoptes scabiei* var. *vulpes* in a test for *Sarcoptes scabiei* var. *canis* is not thought to of concern as prior studies have demonstrated that varieties of *S. scabiei* share common antigens which are recognised by antibodies from different host species (Arlian 1989, Estes & Estes 1993, Arlian *et al.*, 1996a). The use of a goat polyclonal anti-dog IgG for the detection of jackal IgG may lower the sensitivity of the test if there are significant differences between dog and jackal IgG antibodies but it is not possible to assess this potential source of error in this study.

In this study the level of the antibody and the severity of the disease are positively correlated but this relationship requires further evaluation by correlating the antibody response with the mite burden. Nonetheless, a similar correlation has been seen in dogs in that those with the most pronounced clinical signs of infection had the highest antibody levels and those exhibiting only slight clinical signs showed only a small increase in the OD values (Bornstein 1991, Bornstein & Zakrisson 1994).



The ELISA may not be a useful tool for the detection of past exposure as IgG antibodies in beagles were only detectable for 1 to 4.5 months after they appeared (Lower *et al.*, 2001). Furthermore, the test cannot be used immediately post infection as antibodies in dogs may take up to 5 weeks to develop (Scott *et al.*, 2001). Nonetheless, the ELISA is capable of detecting exposure in wild jackals and should be used in conjunction with skin biopsies for the diagnosis of infection, in this and other wild canid species. Further studies should explore the use of this test in other wildlife species. Diagnostically, the results of this field evaluation show that this ELISA is still more informative than skin scrapes alone which are informative in less than 50% of cases (Griffin *et al.*, 1993, Bornstein *et al.*, 1996, Scott *et al.*, 2001), despite use of mange lesions as evidence for infection.

#### 5.6.1.2 *Canine parvovirus type 2*

The low level of exposure in jackals is surprising for a number of reasons. First, this pathogen is likely endemic in the coastal dog population as indicated by the high and stable seroprevalence (and an  $R_0$  well above 1). Second, the persistence of the virus in the environment for long periods of time (Pollock 1982, Gordon & Angrick 1986) greatly increases the chances of transmission relative to a pathogen such as CDV which is far less stable in the environment. The effective transmission of CDV between jackals and dogs and the fact that they are both seropositive to all the other pathogens tested indicates that a number of different transmission routes, with different effective contact rates, may exist between these two species.

High seroprevalences in other studies (Alexander *et al.*, 1994, Sharmir *et al.*, 2001) indicate that all three jackal species are capable of forming detectable humoral immune responses to the virus and the HAI test has been used on jackal sera in another study with success (Alexander *et al.*, 1994). Only 16 individuals are required (assuming a population size of 160 individuals) to estimate a seroprevalence of 1.1% to within a 95% confidence interval (Win Episcopo (Blas *et al.*, 1998)). It is not possible to draw any firm conclusions from the histopathological changes observed in the GI tracts of the jackals in this study as these changes may also be caused by

other diseases, severe parasitism, toxins or other factors, and not all jackals' GI tracts were examined. Suffice to say that no necrosis was observed and epithelial cell necrosis is one of the changes associated with CPV-2 infection.

The transmission of CPV-2 between sympatric domestic and wild canids may not always occur, even when the potential for transmission exists. Crab-eating foxes (*Cerdocyon thous*) did not show any evidence of exposure to CPV-2 despite evidence of overlapping ranges with a seropositive and infected domestic dog population (Courtenay *et al.*, 2001) and African wild dogs sympatric with seropositive domestic dogs in Namibia also tested seronegative (Laurenson *et al.*, 1997b).

Several factors may explain the limited transmission of CPV-2 to the jackal population. Firstly, the longevity of CPV-2 in the environment may be reduced in a high UV desert environment. Secondly, only young infected dogs actively secrete virus in an endemic situation, and a high proportion of these die thus limiting the opportunities for transmission. Thirdly, of the jackals that reach the towns, few may return to the colony as they are normally shot on sight. Fourthly, of those which do not perish in the urban areas, only a fraction will come into contact with pups during their short infectious period or with contaminated adults. Seasonal factors may also limit parvovirus-transmitting contacts if jackals are less likely to enter towns when food availability at the colony is high. A combination of these factors may require a high proportion of the domestic dog population to be infectious, as occurred during the CDV epidemic, in order for spill-over to occur.

If in the future the spill-over of CPV-2 into the jackal population does occur, it is likely to spread rapidly through this naïve population resulting in a decline in the coastal jackal population with mortality in all age classes. But CPV-2 would likely eventually become endemic in this population after which mortality would be restricted to pups. Hence the long term effect of CPV-2 in the jackal population would not likely be significant.

### 5.6.1.3 Canine adenovirus type 1

The high and stable seroprevalence and an  $R_0$  of above 1 in both species indicate that CAV-1 is likely endemic in jackals and dogs. But the force of infection is likely greater in the jackal population as indicated by the larger  $R_0$  and the absence of an age-seroprevalence trend across the jackal age classes as seen in the domestic dogs. The high jackal population density likely facilitates the transmission of this highly contagious disease as the seroprevalence in other jackal populations (Table 5-15) is considerably lower (Spencer *et al.*, 1999). Comparable seroprevalences have been detected in some wolf populations (Stephenson *et al.*, 1982, Zarnke & Ballard 1987) but other wolf populations and species such as coyotes have much lower seroprevalences (Johnson *et al.*, 1994, Cypher *et al.*, 1998) (Table 5-14).

**Table 5-15: A comparison of the seroprevalence of CAV-1 in wild canids.** The exact binomial 95% confidence intervals were calculated from data provided in the respective references. 'NP' calculation of 95% CI not possible as number of seropositive individuals not provided.

Species	Seroprevalence % (n)	95% CI	Selected references
<i>C. mesomelas</i>	94.3 (88)	87.2-98.1	This study
<i>C. mesomelas</i>	9.1 (22)	1.1-29.2	(Spencer <i>et al.</i> , 1999)
<i>C. adustus</i>	37.5 (16)	15.2-64.6	(Spencer <i>et al.</i> , 1999)
<i>C. lupus</i>	94.7 (57)	85.4-98.9	(Stephenson <i>et al.</i> , 1982)
<i>C. lupus</i>	82.8 (87)	73.2-90.0	(Zarnke & Ballard 1987)
<i>C. lupus</i>	36.8 (19)	16.3-61.6	(Johnson <i>et al.</i> , 1994)
<i>C. latrans</i>	68 (152)	NP	(Cypher <i>et al.</i> , 1998)

Clearly the seroprevalence of CAV-1 varies greatly between species and populations and this may be due, in part, to the different social systems and hence the behaviour of the different species e.g. scent-marking. The high prevalence in this study may also be due to prolonged secretion of the virus from jackals living at high densities and the use, by this species, of urine for scent marking. Domestic dogs which survive infection may secrete virus for extended periods of time as CAV-1 can persist in the kidneys far longer than in any other organ with virus excreted in the urine for at least 6 to 9 months after infection (Greene 1998).

The observed age-seroprevalence pattern in jackals may be due to exposure at a young age resulting from the high population density and high prevalence of infection and a long-lived immunity. Immunity in wild canid species may be durable, if not life-long, as a high degree of immunity has been achieved experimentally in foxes (Green *et al.*, 1930, cited in Woods, 2001) and domestic dogs are immune for life.

The post-mortem and histopathological changes observed in the jackals are very similar to those seen in the liver and kidneys of domestic dogs affected by CAV-1, with signs including acute hepatitis, chronic hepatic fibrosis or focal interstitial necrosis (Greene 1998). The focal necrosis of the liver seen in the jackal cases, is similar to that seen in dogs with partial immunity that survive the initial stages of infection and the kidneys of many dogs that recover are studded with the multiple white foci of necrosis also seen in one of the jackal cases (Greene 1998). Therefore, it is unlikely that CAV-1 was the primary cause of death in those jackals with lesions.

It is possible that any CAV-1 infections in jackals were exacerbated by concurrent infection with CDV as this is known to occur in dogs with CD (Greene 1998). There would have been opportunities for co-infection as co-exposure to CDV and CAV-1 occurred in 48.9% ( $n=88$ ) of jackals (95% CI 38.1-59.8) and in 36.3% ( $n=80$ ) of dogs (95% CI 25.8-47.8). Although the post-mortem and histopathological changes observed in the jackals are consistent with CAV-1 infection, further testing would be required to confirm infection as individuals were only tested for exposure to the virus and inclusion bodies were not detected.

The effect of CAV-1 infections on wild canid populations is unknown but the long term-effects are thought to be negligible (Woods 2001); in coyotes mortality could not be attributed to CAV-1 infection (Holzman *et al.*, 1992). It is therefore unlikely that, having attained such a high seroprevalence, that CAV-1 is having a serious impact on the coastal jackal population.

#### 5.6.1.4 *Helminth infections*

As may be expected, the results of the faecal analysis reflect the predominance of the marine component of the jackal's diet (Stuart 1976, Hiscocks & Perrin 1987, Nel *et al.*, 1997) with a high prevalence of *Diphyllbothrium* eggs which is a parasite of marine mammals and birds. This survey has also highlighted the high prevalence of helminth eggs and protozoan oocysts in this jackal population (74.1%,  $n=35$ , 95% CI 53.7-85.4), and the potential for the transmission of zoonotic infections, namely *Toxascaris leonina* and *Diphyllbothrium spp.* which are of concern to humans in South Africa and elsewhere (Minnaar *et al.*, 1999, Minnaar *et al.*, 2002, Raether & Hanel 2003, Torres *et al.*, 2004). But the involvement of the jackals in this study in the transmission of zoonotic intestinal parasites will require the identification of the nematode, Taenid, Acanthocephalan, protozoan oocysts and unidentified eggs in a more accurately quantitative study. It would also be of interest to perform comparative studies of the jackal and dog populations of the coast (and elsewhere in Namibia) as jackals could potentially act as a source of re-infection of zoonotic parasites for domestic dogs.

#### 5.6.1.5 *Canine herpesvirus*

It is not possible to conclude if this pathogen is endemic in the jackal population as only a small subset of samples were tested for exposure. The difference in seroprevalence between jackals and dogs is likely due to the difference in intra-specific contacts, the higher density of the jackal population augmenting the spread of the disease.

#### 5.6.1.6 *Dirofilaria immitis*

Concurrent infections with *Dirofilaria immitis* and canine ehrlichiosis have been detected in other domestic dog populations (Gothe 1999, Tarello 2002). The presence of the zoonotic heartworm, *D. immitis*, in domestic dogs may be due to infection

from a mosquito vector on the coast, translocation of the dogs from infected areas inland, or the movement of infected mosquitoes to the coast. Because *D. immitis* can, via mosquitoes, infect humans and cause pulmonary and extra-pulmonary dirofilariasis (Skidmore *et al.*, 2000, Kim *et al.*, 2002, Rodriguez *et al.*, 2002, Tsung & Liu 2003) its presence in domestic dogs is of concern. Studies have reported infection with *D. immitis* in mosquitoes and dogs of urban areas (Cancrini *et al.*, 2000, Cancrini *et al.*, 2003). The risk posed to the human population on the coast must therefore be assessed by the screening of the domestic dog and jackal populations and the testing of mosquitoes captured from the same locations.

#### 5.6.1.7 Conclusions

Although evidence of the effect on mortality at the population level is hard to obtain, it is likely that the individual pathogens in this study do not have a significant effect on the long term persistence of the jackal population, although combined, they may act to reduce the population size. All the pathogens considered in this study are only likely to significantly affect adult survival when first introduced into the population, and once endemic, mortality is likely restricted to the youngest age classes. As indicated by the population viability analysis of the African wild dog, pathogens which result in significant adult mortality, such as rabies, will negatively affect population persistence whereas pathogens which kill only pups, such as CPV-2, had weaker effects on population persistence (Vucetich & Creel 1999).

Clearly, not all the pathogens introduced into this jackal population will become endemic. The low turnover and the high density make prolonged excretion (in adults), and/or persistence in the environment a requirement for long-term establishment. Furthermore, the biology and social structure of the jackal population will result in very different patterns and levels of exposure than those in domestic dogs, as seen for diseases such as CHV and CPV-2.

But the high rates of infection (for all pathogens except CPV-2) and the low levels of mortality, relative to the year of the CD epidemic, indicate that there is widespread

natural immunity in this jackal population which will aide pathogen persistence and therefore the potential for spread to other species which come into contact with the jackal. It is clear that jackals are capable of expanding the host and geographical ranges of most, if not all, the pathogens typically found in domestic dog populations. Spill-back into the domestic dog population is also likely and further studies should, in addition to proving infection with the pathogens, consider if control strategies for the dog population should take re-infection from the jackal population into account.



## **Chapter 6: Contacts for disease transmission**

## 6.1 Abstract

Epidemiological models of disease persistence used to further our understanding of disease dynamics and to evaluate the outcomes of different control strategies are very sensitive to the parameter describing the rate of contact between individuals. Estimates of this key parameter, the contact rate, are often based on averages and assumed to be constant for all members of the population. Evidence of heterogeneity in contact rates between subsets of a population does not support the use of a single average rate and this may generate erroneous model outputs. This study investigated the occurrence of contacts between jackals and seals, heterogeneities in contact rates, and mixing patterns in the jackal population of Cape Cross using behavioural observations and radio-telemetry data. CDV-specific contact rates were calculated from detailed behavioural observations of over 500 individuals. Jackals made an estimated 11 contacts per day with seals and the frequency distribution of jackal-jackal contacts was highly aggregated with 23% of jackals responsible for 80% of all the contacts. The contact rate for male jackals was roughly 2.5x higher than that of the rest of the population. The total numbers of contacts were compared for different subsets of the jackal population and male jackals were found to make significantly more contacts than the rest of the observed population ( $\chi^2_1=7.37$ ,  $P\text{-value}=0.007$ ); there was no significant difference for jackals with clinical signs of CDV infection ( $\chi^2_1=0.05$ ,  $P\text{-value}=0.824$ ). Radio-telemetry data indicated a high degree of overlap in home range at the seal colony and this provides considerable potential for mixing between individuals. This study provides a detailed example of a complex system with a high rate of contact within and between carnivore species, jackals and Cape fur seals, and evidence of heterogeneity in rates of contact within the jackal population. The results provide insight into why exposure to CDV was high relative to other studies. This study highlights the need for more than one estimate of contact rate within populations in epidemiological models.

## 6.2 Introduction

The rate and patterns of contact between infectious and susceptible individuals in a population is fundamental to the persistence and dynamics of pathogens. At a most basic level, there is a minimum level of contact between infectious and susceptible members of a population required to sustain an epidemic. Kendal's threshold theorem states that an epidemic will occur if an infectious individual makes contact with, and transmits disease to, more than one susceptible individual (Bailey 1975). In other words, the basic reproductive rate of a pathogen ( $R_0$ ) must be greater than 1 and the formula for the basic reproductive rate of a macroparasite, defined as the number of infected individuals arising from one infective individual introduced into a population of susceptibles (Equation 1.0, Figure 6-1), includes a measure of contact rate (Anderson & May 1991).

The contact rate is a key parameter in mathematical models of disease persistence which are used to describe pathogen dynamics and to investigate the effects of different control programmes. Hence the estimate of contact rate can affect the application of control strategies such as vaccination. The proportion of a population,  $p_c$ , which must be vaccinated in order to eliminate infection may also depend on the contact rate as it can be calculated from the  $R_0$  of the pathogen (Equation 2.0, Figure 6-1) (Anderson & May 1991).

Because of the importance of contact rate in the transmission dynamics of a pathogen, mathematical models of disease transmission are very sensitive to changes in contact rate in that too high a contact rate may result in the crash of both the pathogen and the host and too low a contact rate in the extinction of the infection in a population (Macdonald & Bacon 1982). But one of the most frequently violated model assumptions is that all individuals in a population are the same (Anderson *et al.*, 1981, Rhodes *et al.*, 1998, Hawkins *et al.*, 2002). In other words, some models assume the contact rate is equal for all the individuals in a population. But there is an abundance of evidence which indicates that contact rates, in both human and animal populations, do vary so although the 'single contact rate' approach may be sufficient to obtain general insights into disease persistence in a population, excluding

variations in contact rate between subsets of a population may lead to an underestimate of  $R_0$  and as a consequence, erroneous control policies (Hawkins *et al.*, 2002).

**Figure 6-1: The relationship between the basic reproductive rate of a microparasite and the contact rate.**

$$R_0 = \frac{\beta C(N)}{(\alpha + b)} \quad \text{Equation 1.0}$$

The basic reproductive rate of a microparasite,  $R_0$ , is dependent on  $\beta$ , the rate of pathogen transmission,  $C(N)$ , the contact rate between individuals and the disease-induced and natural mortality rates,  $\alpha$  and  $b$ , respectively. The percentage of individuals which must be vaccinated in order to eliminate infection from the population can be calculated from  $R_0$  as shown in Equation 2.0.

$$p_c = \left(1 - \frac{1}{R_0}\right) \times 100 \quad \text{Equation 2.0}$$

(Anderson & May 1991)

Quantitative studies of human contact networks have shown how specific aspects of contact patterns can alter the spread of infectious diseases such as sexually transmitted infections (STIs), measles and rubella (Wallinga *et al.*, 1999). Heterogeneity in sexual behaviour greatly affects the transmission dynamics of STIs such as the human immunodeficiency virus (HIV) (Anderson 1991). Measles and rubella primarily affect younger children and so age-specific contact rates are a crucial factor in determining the design of control programmes (Anderson & May 1985, Grenfell & Anderson 1985).

In the case of wild animals, the evidence is harder to gather, but behavioural ecology studies have demonstrated heterogeneity of contact behaviour in wild populations. A review of field studies found that male mammals and birds had significantly higher prevalence of worm infections than females (Poulin 1996), suggesting that behavioural differences may lead to differences in rates of exposure. Differences in

grooming rates between male and female impalas result in higher densities of ticks being harboured by males (Mooring 1996) which may therefore suffer a higher level of exposure to tick-borne diseases. Male cats are thought to be primarily responsible for the transmission of feline immunodeficiency virus which is transmitted exclusively through bites (Fromont *et al.*, 1997, Courchamp *et al.*, 1998). The application of Pareto's Principle (Pareto 1906), which states that 20% of people own 80% of the wealth, to disease transmission in humans and wildlife, has shown that for the transmission of a range of pathogens, approximately 20% of the population is responsible for 80% of transmission (Woolhouse *et al.*, 1997).

Population subsets of interest to studies of disease transmission are those which are defined by factors which have the most pronounced effects on contact rates. Hawkins *et al* (2002) list a number of key variables which will affect a range of behaviours, including age and gender, which can affect, amongst other factors, group size, aggression, territoriality, greeting behaviour, grooming, the number of mates, dispersal, migration and daily ranging behaviour and feeding behaviour. Hence the different subsets of a population, such as sex and age classes, will exhibit different behaviours and patterns in contact rates. It has been proposed that it is the behaviours which vary significantly between these subsets which are important in epidemiological studies (Hawkins *et al.*, 2002).

Contact rates will not only vary between subsets of a population, but between the same subsets of different populations of the same host species. This variation is a result of the adaptive relationship between a species's social system and its ecological circumstances (Macdonald 1979). Food availability and human disturbance, for example, will affect a population's social organisation as well as individuals' home ranges and movements. Hence different habitats will result in intra-specific differences in social structure and population density and therefore contact rate.

Such intra-specific variation in social organisation is very pronounced in wild canids. Of these species, it is the opportunistic carnivores, such as the jackal, which are

especially flexible in their social organisation, as demonstrated by a number of behavioural ecology studies (Lamprecht 1978, Yom-Tov 1994, Apps 2000, Macdonald 2001, Atkinson *et al.*, 2002, Loveridge & Macdonald 2002). As an indication of variation in social organisation and contact rates, home range varies greatly within all three of the jackal species. The range size of adult golden jackals varied from 8 km<sup>2</sup> to 48km<sup>2</sup> in the farmland in Ethiopia (Admasu *et al.*, 2004), to 2km<sup>2</sup> in Kenya (Fuller *et al.*, 1989), and 1-3km<sup>2</sup> in the Serengeti (Moehlman 1983) and to less than 1km<sup>2</sup> in Israel (Macdonald 1979).

The black-backed jackal shows similar variation in home range size (Table 6-1). As described by the Resource Dispersion Hypothesis, food distribution and availability are factors which determine home range size and therefore contact rate. For example, coyotes living at a waste disposal pit had smaller home ranges and formed groups whilst those feeding outside of the waste disposal area had much larger home ranges (Hidalgo-Mihart *et al.*, 2004). Hence dispersed resources will result in larger home range sizes whilst clumped resources, such as a seal colony or rubbish dump, lead to a break down in territoriality in jackals and the formation of groups (Macdonald 1979, Hiscocks & Perrin 1987, Hiscocks & Perrin 1988). These differences in group formation and home range size translate into variations in the probabilities contact and therefore of disease transmission. Within groups, the probability of transmission of pathogens is high – the probability of contracting rabies in a group is estimated at 100%, whilst the chances of transmission between groups is much lower (White *et al.*, 1995).

**Table 6-1: Home-range sizes of black-backed jackals in different habitats.**

Area	Habitat type	Home-range size (km <sup>2</sup> )	Sample size	Reference
Cape Cross, Namibia	Protected area	24.9	4	(Hiscocks & Perrin 1988)
Suikerbosrand Nature Reserve, South Africa	Protected area	24.8	10	(Ferguson <i>et al.</i> , 1983)
Kalahari Gemsbok National Park, South Africa	Protected area	5.3	8	(Ferguson <i>et al.</i> , 1983)
Transvaal farmland, South Africa	Farmland	181.7	9	(Ferguson <i>et al.</i> , 1983)
Giant's Castle Game Reserve, South Africa	Protected area	18.2	10	(Rowe-Rowe 1982)

Variation in contact rate between the subsets of a jackal population is also evident as there are significant differences in behaviour between the breeding and non-breeding individuals within jackal populations. Sub-adult jackal home ranges overlap with conspecifics more than those of paired adult jackals whose home ranges are smaller and mutually exclusive and young jackals are more likely to disperse than adults (Ferguson *et al.*, 1983, Moehlman 1983, Fuller *et al.*, 1989). Hence the use of a single contact rate value or mean for a species in models of disease transmission, given the wide variation in home range size within a species, would provide a poor predictor of the disease dynamics and limit the use of the model's predictions to one population.

Another important consideration, in addition to that of contact rate heterogeneity, is the definition of a contact. Two individuals can make a number of contacts not all of which will transmit disease. It is therefore important to measure contact rates according to the primary transmission routes of the pathogen(s) in question. The contact rates that will transmit a sexually transmitted disease such as CHV will be very different to those for CDV which is transmitted predominantly via aerosol and therefore requires only close proximity and not intercourse. Incorporating pathogen-specific contact rates in mathematical models will improve the accuracy of the predictions as demonstrated by Berthier *et al.* (2000) who modelled the transmission



of feline panleukopenia virus using a model which incorporated two transmission modes, one for direct contact between individuals, and one for indirect transmission by contact between cats and the contaminated environment. This model showed agreement with the observed trends and was able to explain why the introduction of the virus failed to control the cat population (Berthier *et al.*, 2000). Hence difficulties in explaining observed disease patterns may be resolved with the consideration of pathogen specific contact rates stratified by appropriate population subsets such as age classes, sexes or even infection status if infection significantly alters an individual's behaviour.

Contacts between individuals, and therefore the spread of disease, are dependent on a number of behavioural factors such as grouping, individual behaviour and dispersal behaviour, but such behavioural factors have received little attention from wildlife epidemiological studies (Caro 1999). This is likely due to the difficulties associated with collecting contact information and data on individual movements of wild animals in free-ranging populations, or generalising such information across populations. The WAIFW (whom acquires infection from whom) matrices used in studies of human diseases (Anderson & May 1985, Anderson & May 1991, Edmunds *et al.*, 1997) can be applied to animal systems (Dobson 1995) but they require very comprehensive and detailed datasets based on actual observation of contacts between individuals of known age, sex and/or infection status.

As a consequence, disease transmission and studies of transmission between wildlife species frequently rely on proxy measures of contact such as home range or area overlap as determined from radio telemetry studies (Rhodes *et al.*, 1998, Courtenay *et al.*, 2001). But practically, it is very difficult to accurately predict contact rates from home range overlap, especially when estimated from radio-telemetry data (Bekoff & Wells 1981). One cannot know, without observing the animals directly, if two individuals actually make contact within the overlapping area. Even when home range overlap is extreme, there is no guarantee of frequent contact, and when resources are patchy and shared among animals, contact rates may be high even with low home range overlap. In a study where home ranges overlapped by 54%, jackals

were sighted alone on 87% of occasions (Admasu *et al.*, 2004). In addition, a recent comparative study of animal home range size in relation to body mass argued that overlap increased with size because of an inability to defend home range boundaries at large spatial scales – in other words, high home range overlap indicated low rates of contact between neighbours (Jetz *et al.*, 2004). Loveridge *et al.* (2001), in a study of interactions between jackals for the transmission of rabies, estimated contact rates using radio-telemetry data. The data were analysed using a static interaction analysis which is based on measuring the intensity of use of shared 50x50m cells of the study area. But this requires a high level of accuracy in timing and location of multiple individuals which may not always be possible using radio telemetry. Studies of contact rate using telemetry are also limited by the numbers of individuals which can be radio-collared and tracked. Although some studies may be able to compensate for this by surveying the study area for the numbers of individuals with which the focal animal (radio-collared individual) may come into contact with and adjusting the contact rate accordingly (Courtenay *et al.*, 2001).

## **6.3 Aims**

This study aims to provide evidence for contacts between jackals, between jackals and seals, and jackals and domestic dogs for the transmission of CDV and to answer the following questions:

1. Are there strong heterogeneities in contact rate among subsets of the population?
2. Might these patterns help us to understand the observed prevalence of exposure in jackal and seal populations? Or, do the observed levels of exposure simply reflect the overall average contact rate?

The data will be obtained from behavioural observations of tagged and radio-collared and other unidentified individuals, and from analyses of jackal home range size and overlap. The importance of collecting detailed contact rate data will be assessed and this study will be used to make practical recommendations for future epidemiological studies of wild canids.

## **6.4 Materials and methods**

### **6.4.1 Behavioral observations for contact rates**

Five locations, covering the northern most 2km of the Cape Cross seal colony, were selected as observation points because of their accessibility and suitability for observations. Observations were conducted between the 19<sup>th</sup> of July and 6<sup>th</sup> of October 2002, from a vehicle over two four hour periods starting at dawn (usually at about 6am) and 15:00. These periods were selected to cover as much of the jackals' activity periods during daylight hours as possible. The observation points were selected at random, and individuals for the focal observations were selected, irrespective of their activity, by scanning from left to right or vice versa (the direction of scan was also selected at random) across the field of view until a jackal was encountered. The jackal was then followed for as long as it was visible.

Behaviour was recorded using a Psion Workabout (Psion Teklogix Ltd., UK) with a programme written to record the start and end time of each focal follow, the number of the individual (sequential from the first individual followed in the observation period), the observation position and the type of contact, and the time at which the contact occurred. Jackals were recorded as diseased or non-diseased according to whether or not they had visible neurological signs (which were assumed to be caused by infection with CDV). Jackals in each disease category were also identified as male or female where possible, relying on ear-tags, radio-collars or simply visual identification. If an individual could not be identified as male or female it was recorded as unidentified. Contacts were recorded as either direct between two or more individuals or as indirect (via a carcass for example), and contacts with individuals showing neurological signs of CDV infection, were additionally noted. In between focal follows of individuals, observers scanned the area for groups of jackals, defined as two or more individuals engaged in the same activity, and recorded group size.

An ethogram was developed to include all the contacts which may transmit CDV, directly or indirectly (Table 6-2). CDV is transmitted predominantly via aerosol or

droplet contact as it is most abundant in the respiratory exudates, however, it can be isolated from most other body tissues and secretions including urine and it will persist in body tissues for up to 3 hours at 20°C (Greene & Appel 1998). Hence oronasal, excretory and carcass contacts were also used to calculate a CDV-specific contact rate.

**Table 6-2: The ethogram for the transmission of CDV between jackals and between jackals and seals.**

Contact	Description
Close proximity to a seal	Within 1 meter of one or more seals but no physical contact made
Seal bark	Seal barking in jackal’s face at a distance of under 1 meter
Close proximity to a jackal	Within 1 meter of one or more jackals but no physical contact made
Naso-naso	Oronasal contact, typically sniffing of mouth and nose
Naso-anal	Sniffing of anal area
Bite	Closing of jaws on any area of the body of another jackal
Grooming	Grooming of another jackal by ‘nibbling’ head, neck and shoulder areas
Sniffing urine	Sniffing the area where another jackal was seen to urinate
Eating scraps or carcass	Eating scraps or feeding off a carcass
Eating scraps or carcass of another jackal	Eating scraps or feeding off a carcass seen to have been eaten by another jackal
Urinate	
Defecate	

Observations of contacts between the focal jackal and another jackal were limited to recording the number of contacts only as it was not possible to distinguish whether contacts were made with the same or different individuals. In order to improve the accuracy of the observations only jackals which were followed for 1 minute or more were used in the calculations of contact rates. The cut-off point of 1 minute was calculated as approximately half the mean interval between contacts.

#### 6.4.1.1 Average duration of resting periods during activity periods

If an individual started resting during the focal follow the time spent resting was recorded. If the individual remained resting in the same position for longer than 20 minutes, another jackal was selected for observation but the resting individual was checked visually every few minutes so that the time at which the resting period ended could be recorded. The resting period was judged to be over when the jackal got up and moved away and no more than 2 individuals were followed at the same time. The average time spent resting was calculated for 50 individuals selected at random from the dataset.

#### 6.4.1.2 The distemper-specific contact rate for jackals

An average daily distemper-specific contact rate per jackal was calculated as this is the type of data which would normally be used in a mathematical model. The average contact rate between jackals was calculated by summing the total numbers of the different types of contacts made by each jackal and dividing this number by the total number of jackals followed to get the average number of contacts per jackal. The average number of contacts per jackal per hour of activity period was calculated by dividing the average number of contacts per jackal by the average duration of the focal follow (to obtain the contact rate per minute) and multiplying this value by 60 to obtain the contact rate per hour of activity period. The average number of contacts per jackal per 24hr period was obtained by multiplying the average number of contacts per jackal per hour of activity period by the total activity time. The total activity time was calculated by subtracting the average time spent sleeping during observations from a total activity period of 8 hours (Ferguson *et al.*, 1988, Kaunda 2000). The contact rate was calculated for each subset of the jackal population which could be recognised visually (e.g. male, female, tagged or collared individuals of known ages, individuals with clinical signs of CDV infection).

#### 6.4.1.3 The distemper-specific contact rate for jackal-seal interactions

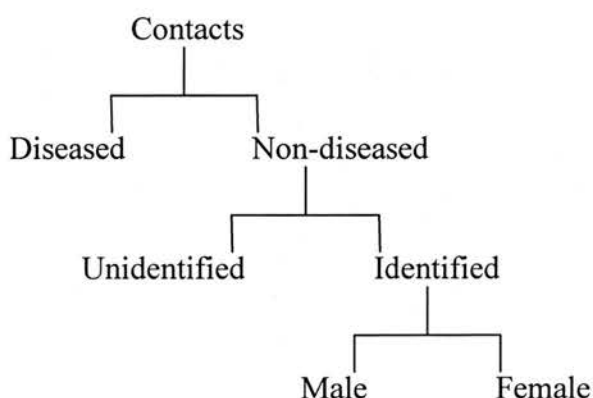
An estimate of the distemper-specific contact rate between jackals and seals was calculated as for jackal-jackal contact rates described above but only from the total number of aerosol contacts which consisted of seals barking in the jackal's face and jackals being within close proximity of a seal (Table 6-2).

#### 6.4.1.4 Statistical analyses of jackal-jackal contacts

In order to investigate the potential for heterogeneity in contact rates the total number of distemper-specific contacts made between jackals was summed and the percentage of the total number of individuals observed responsible for 80% of the total number of contacts calculated (Woolhouse *et al.*, 1997).

Generalised linear models (GLMs) (Crawley 1993) were used to investigate differences in the numbers of contacts made between the subsets (sex and disease status) of the jackal population. Due to the aggregated frequency distribution of the contacts between jackals, a negative binomial error distribution was selected for the analyses. It was necessary to analyse the data in a hierarchical manner (Figure 6-2) due to the fact that males and females could not be identified in the diseased subset.

**Figure 6-2: The hierarchical structure of the analysis of jackal contact data.**





Firstly, a GLM was used to test whether the numbers of contacts made by jackals with and without neurological signs of CDV infection (diseased and not-diseased) were significantly different. The non-diseased fraction of the data set was then split into identified and unidentified individuals according to whether or not the sex had been identified for each individual jackal. A second GLM was then used to compare the unidentified and identified (male or female jackals) subsets to determine if jackals in these subsets made significantly different numbers of contacts; this comparison was used to check for possible biases in the data. In order to determine if there was a significant difference between male and female jackals, the recognised subset was then divided into male and female individuals and a third GLM used to test if sex was predictive of the number of contacts. But due to the small number of females recognised ( $n=4$ ) there were concerns about the validity of the results. Hence the females were re-classified as unidentified and a fourth GLM used to test if males and unidentified individuals made significantly different numbers of contacts. If there was a significant difference between males and unidentified jackals, the result was confirmed by re-classifying males as unidentified and comparing all identified females against the unidentified class.

All analyses were performed in SPLUS (SPLUS 2000, Mathsoft Inc.) and the adequacy of each GLM model checked by comparing the residual degrees of freedom with the residual deviance. Counts of contacts were classed as integers and all predictor variables as factors with two levels (i.e. diseased, not diseased; unidentified and identified; male and female; male and unidentified; female and unidentified).

Ideally the analysis would have taken day of observation into account as a random effect but it is not possible at present to apply a mixed effect model to data with a negative binomial error structure. The total observation time for each day could also not be incorporated into the models as the numbers of contacts were dependent on the total time of observation. The conversion of the counts of contacts into a contact rate by dividing by the total observation time would have resulted in a contact rate

with a negative binomial distribution when this type of distribution assumes the data is counts of occurrences and not a rate.

## **6.4.2 Radio-tracking**

### **6.4.2.1 *Radio collars***

Sedated jackals were fitted with nylon collar-mounted transmitters (Biotrack Ltd., Dorset, UK; 173.0-173.3 MHz, 200g) as described in Chapter 2 (section 2.4.2.2). The transmitters were detectable at approximately 2 km and had an estimated field life of approximately 18 months. The weight of the collars was below the recommended collar to body mass ratio of 3% (Cypher 1997) as the average weight of radio-collared jackals was 10.4 kg ( $n=13$ ).

### **6.4.2.2 *Radiotracking***

Jackals were tracked by 2 volunteers by taking fixes every 20 minutes during 4 hour tracking periods (06:00-10:00, 10:00-14:00, 14:00-18:00, 18:00-22:00, 22:00-02:00, 02:00-06:00) with 2 tracking periods per 24 hours. The jackal to be tracked and the tracking period were selected at random with equal numbers of periods per jackal and all periods covered at least once for each jackal; schedules were amended to include newly collared individuals. Volunteers were trained by hiding a transmitter and testing their triangulation accuracy to within 10m.

Because no vehicles were allowed off road and the available tracks were too limited for tracking purposes, jackals were tracked on foot or from mountain bikes. Individuals were located using a flexible Yagi antenna (3 elements, 148-174 MHz, Biotrack Ltd., Dorset, UK) and a Telonics TR-4 receiver (Telonics Inc., Arizona, USA).

### 6.4.2.3 Calculation of fixes from bearings

The signal strength and distance to the collar were determined by placing a helper with a collar at a known distance from the receiver and the signal strength recorded for the known distance. Signal strengths and the respective distances to the jackals are given in Table 6-3. This enabled the estimation of distance to the jackal using the stronger signal strengths thus eliminating the need for constant triangulation.

**Table 6-3: The signal strength and the corresponding distance between the receiver and the transmitter and the respective methods used to calculate fixes.**

Signal strength	Distance to the jackal (m)	Method
Visual	As estimated by the tracker	Single bearing with distance to the jackal
3+	0-300	Two bearings
3	300-600	Two bearings
2+	900-1200	Triangulation
1-2	1200-2000	Triangulation
1	>2000	No fix: move closer to the animal

When the jackal was out of sight and the signal strength less than 3, triangulations were used to determine the animal's approximate location before selecting another site closer where the signal was stronger. Triangulations consisted of 3 bearings taken from three different stations at least 30m apart. If the signal strength was 3 or 3+ and the animal was out of sight, only two bearings were taken to determine the fix, assuming that the animal's location was where the two bearings' trajectories intersected. Fixes for two and 3 location bearings were calculated using Locate II (Nams 1999). If the jackal was visible, single bearings were taken with an estimated distance to the individual, together with the station position. Fixes from single bearings were calculated using basic trigonometry by solving for the opposite and adjacent sides of a right-angle triangle in Excel (Microsoft, 2000). A summary of the methods used to calculate fixes is also given in Table 6-3.

#### 6.4.2.4 *Calculation of home range area*

All fixes were plotted in ArcView Version 3.3 (Version 3.3, ESRI UK Ltd.) using a basic outline of the Cape cross area. The country outline (DCW 1997) map was lacking in some of the geographical detail so that some of the home range fixes appeared to be in the sea. Hence all those fixes within 500m of the coast were acceptable for the calculation of the home range area but those beyond this cut-off were excluded from the analyses and plots of the home ranges. Home ranges were calculated using the minimum convex polygon (MCP) method (White & Garrott 1990) using the Animal movement and Spatial Analyst extensions (Versions 2.0) for ArcView. The harmonic mean method (Dixon & Chapman 1980) was used to remove 5% of outlying fixes, leaving 95% of the fixes for the calculation of each individual's home range.

## 6.5 Results

Behavioural observations used to estimate contact rates were collected over 37 days, comprising 30 morning periods and 28 evening periods. A total of 702 individuals were followed of which 570 were observed for  $\geq 1$  minute, yielding a total of 133 hours of observation. Jackals were followed for an average of 14 minutes (range $\pm$ SD: 1-183 $\pm$ 24) and those individuals which rested during observations spent an average of 24 minutes resting ( $n=50$ , range $\pm$ SD: 1-84 $\pm$ 25). A total of 89 groups were observed with an average size of 4 individuals (range $\pm$ SD: 2-25 $\pm$ 4).

Assuming a total of 8 hours activity per 24hr period and subtracting from this 48 minutes resting time (an average of 24 minutes resting per 4 hour observation period) the average number of contacts per jackal per day (24hrs) was calculated by extrapolating the average contact rate for one hour of activity to 7 hours and 24 minutes. The contact rate between jackals was calculated to be 92 contacts per individual per day.

### 6.5.1.1 *Contacts between jackals*

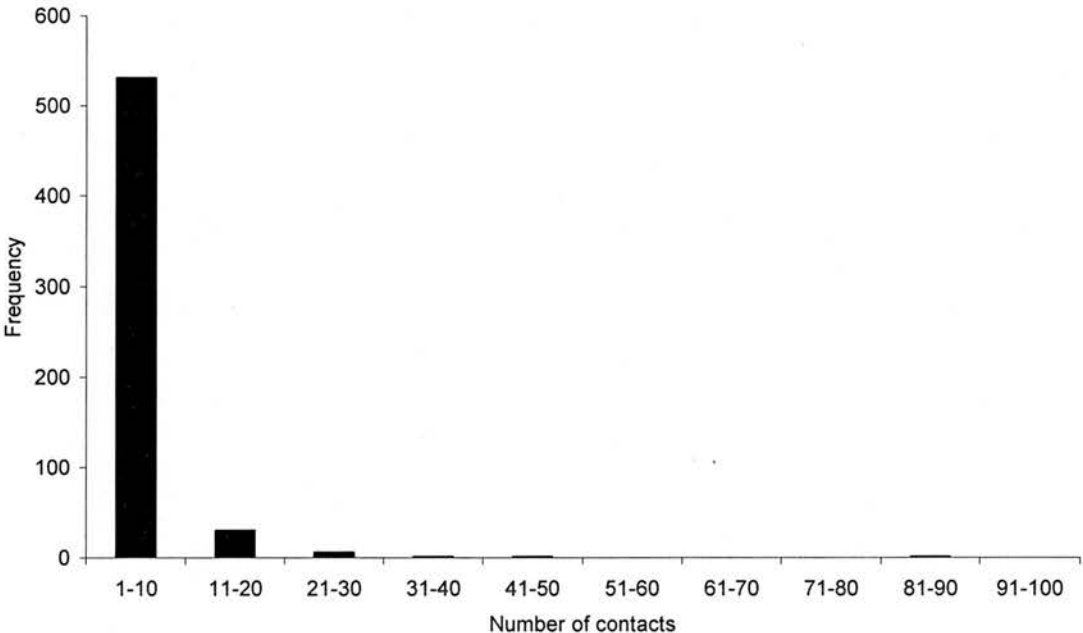
The average daily contact rate between jackals was calculated for the subsets of the population which could be reliably identified during observations. Males were observed more frequently than females: females ( $n=4$  focal observations), males ( $n=31$ ) and those showing clinical signs of distemper ( $n=55$ ). Two radio-collared individuals were included in the observations: one male with 21 focal observations and one single observation of a female. Table 6-4 shows the average distemper-specific contact rates per jackal per day for each subset of the observed jackal population.

**Table 6-4: The jackal-jackal contact rate as calculated for the different subsets of the population.** The rate calculated for 'All jackals' includes all identified and unidentified individuals.

	All jackals	Females	Males	Distemper
Total number of contacts	1704	19	283	167
Number of jackals observed	570	4	31	55
Average number of contacts per jackal	3.0	4.8	9.1	3.0
Average duration of focal follow (minutes)	14.0	12.3	17.1	14.3
Average number of contacts/jackal/activity hour	12.8	23.2	31.9	12.8
<i>Average number of contacts/jackal/24hrs</i>	92	167	229	92

The numbers of contacts made by individual jackals were highly aggregated in a negative binomial distribution (Figure 6-3,  $\phi=0.35$ ) and 23.2% of individuals (132 of 570 jackals) were responsible for 80% of all contacts ( $n=1,704$ ).

**Figure 6-3: Frequency distribution of the distemper-specific contacts made by jackals per focal observation.**



The contact data was divided according to the numbers of hours of observation per day (either 4 or 8 hours i.e. one or two observation periods) and the analyses repeated

for each dataset. The results are presented for both the 4-hour (Table 6-6) and the 8-hour (Table 6-5) observation datasets and although there is no qualitative difference between the datasets in the results, the 8-hour dataset is used for the interpretation of the data as this dataset encompassed more of the observed contacts (72%,  $n=1,704$ ) than the 4-hour data set (26%).

There was no significant difference in the numbers of contacts made by diseased and non-diseased jackals. But males were found to make significantly more contacts when compared to the identified fraction of the jackal population (Table 6-5).

**Table 6-5: The results of the GLM analyses comparing the numbers of contacts made by different subsets of the jackal population.** Analyses of the 8-hour dataset.

Models (explanatory variables)	Residual deviance/residual <i>df</i>	Deviance	<i>df</i>	Coefficient	<i>P</i> - value
Diseased vs. non-diseased	0.99	0.05	1	-0.078	0.824
Unidentified vs. identified	0.98	7.27	1	-0.972	0.007
Male vs. female	1.30	0.721	1	0.680	0.369
Unidentified vs. identified (no females)	0.98	7.37	1	-1.026	0.007
Unidentified vs. identified (no males)	0.96	0.08	1	-0.346	0.779

**Table 6-6: The results of the GLM analyses comparing the numbers of contacts made by different subsets of the jackal population.** Analyses of the 4-hour dataset. 'Deviance'.

Models (explanatory variables)	Residual deviance/residual <i>df</i>	Dev.	<i>df</i>	Coefficient	<i>P</i> - value
Diseased vs. non-diseased	0.94	1.63	1	0.504	0.202
Unidentified vs. identified	0.93	3.87	1	-0.827	0.049
Male vs. female	1.3	0.006	1	-0.074	0.936
Unidentified vs. identified (no females)	0.92	3.27	1	-0.817	0.071
Unidentified vs. identified (no males)	0.90	0.614	1	-0.891	0.433



### 6.5.1.2 *Contacts between jackals and seals*

Jackals made an average of 11 aerosol contacts with seals per day (Table 6-7). The jackals with neurological signs were also seen to make contacts with seals, at an average of 18 contacts per day (Table 6-7).

**Table 6-7: The jackal-seal contact rate as calculated for the different subsets of the population.** The rate calculated for all jackals includes the females, males and distempered jackals.

	All jackals	Females	Males	Distemper
Total number of contacts	195	2	8	33
Number of jackals observed	570	4	19	55
Average number of contacts per jackal	0.3	0.5	0.4	0.6
Average duration of focal follow (minutes)	14.0	12.3	14.7	14.3
Average number of contacts/jackal/activity hour	1.5	2.4	1.7	2.5
<i>Average number of contacts/jackal/24hrs</i>	11	18	12	18

### 6.5.1.3 *Radiotracking data*

A total of 14 jackals were captured and radio-collared at the CCSR, 5 in 2001 and 9 in 2002 (Chapter 2, section 2.10.1.1). Table 6-8 gives a summary of the age and gender of collared individuals and the total length of time for which they were tracked. A total of 3 juveniles, 4 sub-adults and 7 adults, of which 5 were female and 9 were male, were radio-collared over the course of the study.

**Table 6-8: The numbers of jackals of each age class and gender radio-collared at Cape Cross, 2001 and 2002.** The period of tracking refers solely to 2002; \*refers to a jackal with clinical signs of distemper infection.

Jackal ID No.	Month and year collared	Age Class	Gender (M male, F female)	Period of tracking (days)
1	September 2001	Adult	F	15
2		Juvenile	M	20
3		Sub-adult	M	17
4		Adult	M	19
5		Sub-adult	M	0
6	July 2002	Juvenile	F	2
7		Sub-adult	M	52
8		Juvenile	F	29
9	October 2002	Adult	F	17
10		Adult	M	7
11		Adult	M	7
12		Adult*	F	11
13	November 2002	Adult	M	8
14		Sub-adult	M	6

Of those collared in 2001, one could not be located 2 days after release and of the 4 tracked in 2001, only 1 was located in 2002 and this individual's collar ceased to function in October 2002. Of those collared in 2002, two (individuals 6 and 8, Table 6-8) could not be re-located and the disappearance of these individuals from the population is attributed to the epidemic of canine distemper which affected the Cape Cross area in 2002. This limited the period during which individuals were followed as additional individuals had to be radio-collared in subsequent capture sessions in 2002. The adult female (individual 12) with neurological signs of distemper infection at the time of capture disappeared not long after the start of the tracking period before which the neurological signs were seen to worsen.

Difficulties were encountered when attempting to locate individuals outside of the reserve. It was not possible to locate individuals if they left the colony before the start of the tracking period and therefore fixes beyond the immediate area of the reserve were limited. For the same reason it was not possible to record contacts with domestic dogs in the towns of Swakopmund and Henties Bay.

Figure 6-4 shows an outline of the Cape Cross coastline with the MCP ‘home ranges’ for 9 individuals. These are not considered to be realistic home ranges for each individual as the numbers of fixes and the bias for the colony area (with respect to further inland or north or south along the coast) do not allow for realistic home range estimates (Table 6-9) but the MCPs can be used to approximate the range used by each jackal in the colony area. The MCP for the first juvenile male to be captured in July 2002 was not calculated as less than 10 fixes were obtained for this individual (Table 6-9). Two fixes from each of the datasets for jackals 4 and 7 were excluded due to the fact that they occurred too far into the sea, beyond the 500m boundary from the coastline. Although the home ranges extend in different directions away from the seal colony, projecting inland, north and south there is considerable overlap in the immediate vicinity of the colony.

**Table 6-9: The jackal home range estimates with the 95% ellipse area and the number of fixes for each individual tracked in 2002 at Cape Cross.** \*Home range not plotted in Figure 6-10 as only 4 fixes were obtained for this individual.

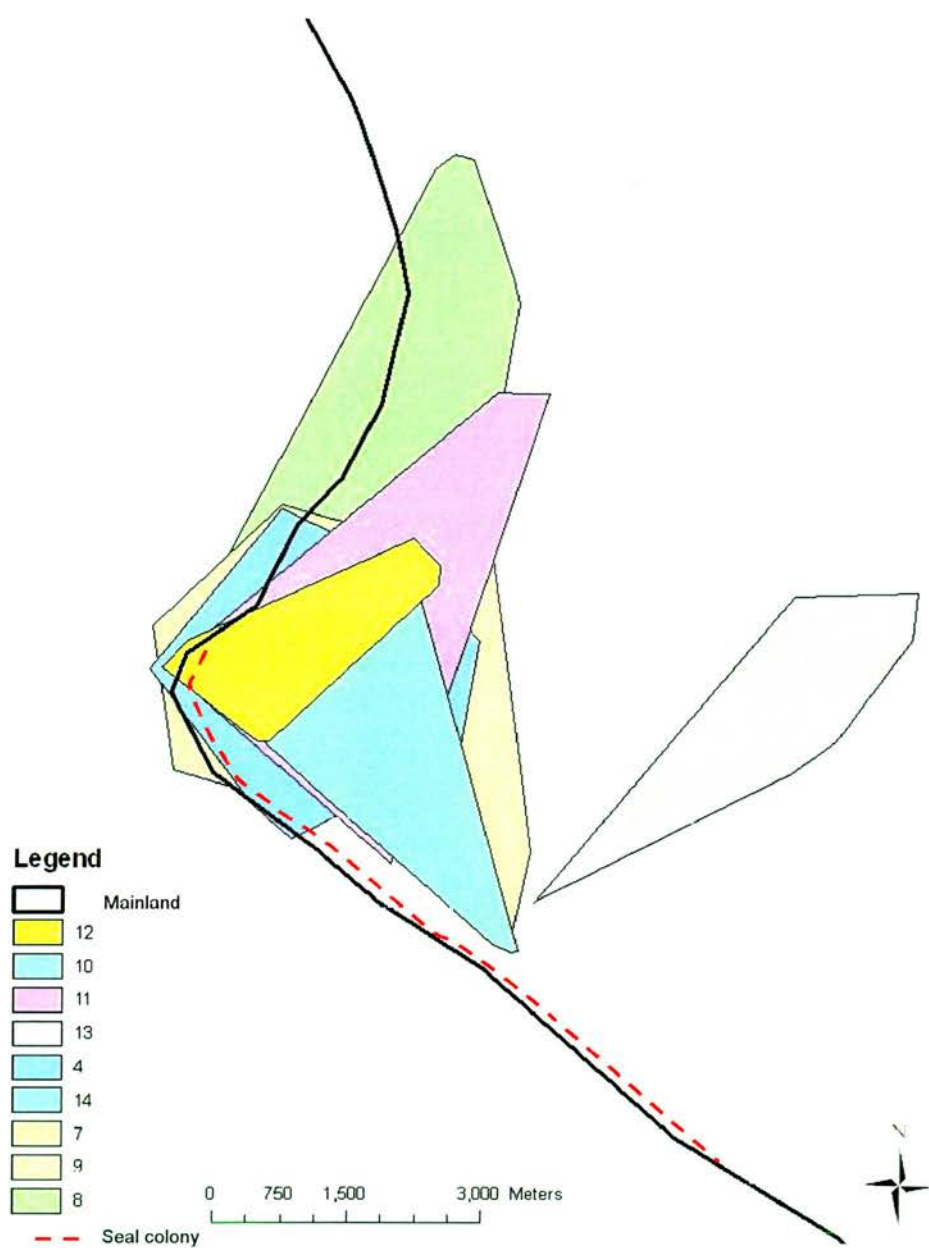
Jackal	Number of fixes	MCP Area (km <sup>2</sup> )	95% Ellipse Area
4	195	8.4	8.1
7	357	6.2	3.8
8	189	16.2	22.3
9	59	9.2	11.7
10	22	5.9	13.9
11	30	9.6	27.0
12	40	3.1	7.3
13	21	4.7	9.8
14	27	2.4	5.3
Average MCP size (km <sup>2</sup> )		7.3	

#### 6.5.1.4 *Contacts between jackals and dogs*

Contact between jackals and dogs was inferred from reports of jackals entering the towns of Swakopmund, from reports of dogs being shot in the Diamond Area, the capture of one individual of a pack of dogs in the Diamond Area and reports of jackals at the rubbish dump and township areas in Lüderitz. Since no records are kept by NamDeb staff (pers. comm. S. Hugo, NamDeb) of the numbers of dogs shot in the

Diamond Area or of the jackals sighted in the town, it was not possible estimate the frequency of contacts.

**Figure 6-4: The minimum convex polygon areas for jackals tracked at Cape Cross in 2002.** A c.8km stretch of the seal colony extending south from the rocky promontory is displayed here.



## 6.6 Discussion and conclusions

### 6.6.1.1 The heterogeneity of contact rates

This study provides the first evidence for heterogeneity in disease-specific contact rates in jackals. Firstly, the difference in the average daily contact rates between males (229 contacts per jackal per day) and the population as a whole (all jackals observed: 92 contacts per jackal per day) proved to be significant (identified subset, excluding females, vs. unidentified:  $P=0.007$ ,  $df=1$ , residual deviance=0.98). Secondly, the frequency distribution of the contacts was highly aggregated such that approximately 20% of the jackal population was found to be responsible for 80% of all distemper-specific contacts which is consistent with the 20:80 rule (Woolhouse *et al.*, 1997). These observations support the existence of a core group of transmitters, some of which may be male.

The observation that males make significantly more contacts than the rest of the population as a whole is perhaps not surprising considering similar findings have been made in other studies (Poulin 1996, Fromont *et al.*, 1997, Courchamp *et al.*, 1998) and a similar bias was seen in the domestic dog population in this study with significantly more males being exposed to CAV-1 (section 5.5.3.1).

The significant difference between the identified and unidentified subsets of the dataset may be taken as an indication of a bias in the data. Biases may be the result of a number of factors, for example observer bias against unidentified individuals but in this case the difference between the identified and unidentified subsets was shown to be due to sex differences in the contact rates. It is possible however, that males were more likely to be observed than females as these are generally less wary than females (pers. obs. S. Gowtage) and were therefore be more likely to occur in the vicinity of the vehicle. Considering the male:female capture ratio of 1:0.85 (Section 2.10.1), more females should have been included in the dataset. There was no significant difference between females and the unidentified jackals but no firm conclusions can be drawn regarding the contacts made by female jackals from these data as the

sample size in the 8-hour data set (females  $n=2$ ) which was used in the analyses was very small.

However, the absence of a difference between diseased and non-diseased jackals may be due to the absence of significant behavioural changes in the distempered jackals rather than a bias in the data as a reasonable sample size was used in the analyses (diseased jackals  $n=27$ ). The aim of the calculation of a separate contact rate for jackals showing neurological signs of CDV infection was to determine if these individuals made fewer contacts than asymptomatic individuals so that the probability of transmission of CDV may be reduced. But the use of jackals exhibiting neurological signs is unlikely to provide a realistic contact rate for infective jackals for two reasons. Firstly, neurological signs in dogs may persist long after the individual ceases to be infective. Secondly, many infections can be asymptomatic and there is great variation in clinical signs, so not all individuals will develop neurological signs (Greene & Appel 1998). But it is difficult to ascertain if infection alters behaviour in a way which reduces the number of disease-transmitting contacts as it is necessary to prove that behaviour is significantly affected during the infective period. If the infective period cannot be reliably associated with visible clinical signs, as in the case of distemper, it may not be possible to obtain a measure of contact rate for the infective subset of the population from behavioural observations.

The contact rates calculated for jackals at this study site are very high compared to other studies. Rhodes *et al.*, (1998) estimated a contact rate for individual jackals of once every 7 days. But this was likely to be an underestimate as it only included contacts between radio-collared individuals. Contact rates in the present study were high because the seal colony represented a concentrated resource and jackals can be highly social. Resource distributions will be a major factor influencing social interactions and contact rates (Macdonald 1979, Macdonald & Bacon 1982) and this study presents a detailed analysis of such a system. It remains to be seen, as more of these types of studies become available, whether it will be possible to develop basic approximations of contact rates (e.g. males make on average twice as many contacts

as females) which can then be generalised across populations of the same species and used to aid our understanding of disease epidemics.

Whilst it is likely that most of the contacts between jackals in this study occurred at the colony, it was not possible to monitor the jackals outside of the colony area. In Chapter 2, evidence from distance sampling transects suggested that jackals made few contacts outside of the colony area. Contact rates between family members within the den (which may be far from the colony), however, are likely to be very high.

#### *6.6.1.2 Home ranges and territoriality*

A high degree of overlap in jackal home ranges was apparent within a small part of the colony area. The 9 MCPs plotted had an average size of 7.3 km<sup>2</sup> and covered a small area of coastline approximately 12km in length. Such overlap implies a high level of mixing between jackals and this was confirmed by the observation of high contact rates.

The MCPs plotted are clearly not representative of the individuals' entire home ranges and although they show a similar degree of overlap, they are smaller than the average home range size recorded by Hiscoks & Perrin (1987) of 29.4 km<sup>2</sup>. Although some jackals may range only within the area of the colony, observations made during this study (tracks and 'highways' extending over 10km inland and dens occurring 7km inland from the colony) and those of Hiscoks & Perrin (1987) show that jackals range beyond the boundaries of the CCSR.

#### *6.6.1.3 Relating observed patterns of contact and exposure*

The breakdown in territoriality of jackal populations at clumped resources (Macdonald 1979, Macdonald *et al.*, 1999) indicates that mixing may be more homogeneous and the *per capita* contact rates high, or in such populations, relative to those from habitats with more dispersed resources. The jackals in commercial



farmland in Zimbabwe are thought to meet only every 7 days (Rhodes *et al.*, 1998) and European red fox, once every 4 to 6 days (Anderson *et al.*, 1981). Certainly in this study, the high density at the colony and the high degree of overlap between home ranges at the colony would have contributed to result in the highest seroprevalence (74.1% at Cape Cross, 2002) for CDV reported in a jackal population to date (see Table 3-1, page 85 for comparisons). Exposure to CDV will also have been augmented by the formation of groups at carcasses in which the probability of transmission likely approached 100%.

One might have expected the seroprevalence of CDV to be closer to 100% considering the high contact rate at the colony but the spread of the disease will have been moderated by a number of other factors. Firstly, the existence of a core group of transmitters and secondly the likelihood that not all contacts between an infective jackal and a susceptible one will result in transmission of the virus. In addition, variation in the duration of the infective period, as seen in dogs (Appel 1969, Appel 1970), will also limit the opportunities for transmission as some jackals will develop a high and effective VN antibody titre and clear the infection before others.

According to Macdonald and Bacon (1982), a high contact rate between susceptible and rabid foxes results in the extinction of the pathogen (and the host) so that an intermediate level of contact is required for a cyclical persistence of rabies in the fox population. The high contact rate may have resulted in a high prevalence of exposure within the jackal population but this did not translate into an increase in the maintenance potential of the population, as evidenced by the rapid spread along the coast and the likely fade-out in 2003 (no symptomatic jackals were captured in 2003 and seroprevalence decreased to 42.1%, see Table 3-13, page 121).

The observation of distemper-specific contacts between jackals and seals provides evidence for the opportunity for the transmission of CDV between these two species. This supports the hypothesis, put forward in Chapter 4, that the spill-over of CDV from jackals to seals may have occurred for a few individuals, but that the spread

through the population did not occur due to the inability of the strain of CDV to result infective in Cape fur seals.

#### *6.6.1.4 Study limitations*

A number of limitations in this study, some a result of the study design and others inherent in the logistical constraints faced in the field, constrained both the data collection and the data analyses. Firstly, the exclusion of 28% of the contacts from the analyses with the use of data only from observation days with 8 hours of observation, limited the power of the analyses as individuals from the male, female and distempered subsets also occurred in the 4-hour dataset. Future studies should therefore aim to have a constant total observation time for each day.

Secondly, it would have been advantageous to include a higher number of identified individuals in the observations, preferably tagged or radio-collared individuals for which the age class, sex and serostatus would have been known. But this would have required the capture and tagging of a larger proportion of the population than was possible in this study. Future studies could, however, focus on the core group of transmitters identified in this study. Thirdly, the view points available for observation which, not being elevated allowed only a limited view of the colony, resulted in numerous short focal observations. This not only introduced unrealistic measures of contact rates into the data, as individuals which were followed for a short time, and which made a number of contacts during that period, had unrealistically high contact rates for the activity period as a whole. Another result of low position of the view points was the limited view of interactions between jackals and seals. Personal observation suggests that the contact rate between jackals and seals is very high, due to the fact that individuals moving through the tightly packed seal colony come into close proximity with a large number of seals. But in the absence of a fuller view of the colony area it was not possible to accurately estimate how many seals a focal jackal came into contact with. Hence an elevated view point would have also provided a more accurate jackal-seal contact rate.

Extending the study period to encompass seasonal changes in contact rate would also be advantageous. Although the study colonies are large, they do not provide a constant abundant food supply. Jackals go through a period of gluttony when the mortality of new-born pups is highest, during the first two months of life in November and December (De Villiers & Roux 1992), after which food supply decreases because jackals struggle to kill larger pups and mortality also decreases with age so that by the following September many of the jackals are in poor condition. The variation in food availability will force jackals to forage further afield, may encourage dispersal and will increase group formation at the colony as jackals fight over carcasses thus altering the contact rates. Seasonal variation in contact rate will also occur as a result of the mating and pupping seasons, indeed, the distemper epidemic in the jackals was likely fuelled by the mating period in June and July (Apps 2000), as discussed in Chapter 3.

Future studies of this population should therefore consider using a raised observation point and focusing on the core group of transmitters for future disease-related behavioural studies. With suitable night-vision equipment it may also be possible to extend the observation periods to cover nighttime activity as this study was only able to capture the 'tail-end' of the jackal's activity periods by observing them during the first and last few hours of daylight.

The radio-telemetry study had two main aims, the first being to provide an indication of the mixing patterns at the colony and further inland, and the second to provide basic evidence of contact between jackals and the urban environment (i.e. domestic dogs). But the radio-telemetry study suffered from a number of limitations, in addition to the loss of collared individuals in the CD epidemic. Firstly, the study was restricted to the Cape Cross area due to the added expense and logistical difficulties associated with locating and tracking animals in amongst the hills and gullies of the Diamond Area in which mountain bikes could not be used and the 4x4 track network is limited. Secondly, because the range of the trackers was limited by the fact that they could not use bikes or vehicles off road it meant that jackals were unlikely to be located if not in the reserve at the start of the tracking session. Hence accurate home

range estimates and evidence of contacts with domestic dogs were not obtained. Future studies would be better off investing in a smaller number of re-usable satellite collars. Technological advances have made these collars not only smaller and more affordable but very accurate in their positioning over large geographical areas and improvements in the analysis of GPS data have further improved the quality of the data (Hulbert & French 2001, Frair *et al.*, 2004). The desert environment of the coast is ideal for these collars as there is no vegetation to interrupt the signal from the satellites. The use of satellite collars may also given an indication of the movement of jackals between colonies. One could consider the jackals at individual seal colonies as large groups because the densities at the colonies can be 10-fold greater than those in between colonies (Nel *et al.*, 1997). A mathematical model of disease transmission in this coastal system would therefore have to include measures of contact between these 'groups' which are likely to be lower than the *per capita* contact rates at the individual colonies.

#### 6.6.1.5 Conclusions and future recommendations

Although the identification of a group of core transmitters may be important and applicable to mathematical models, it may not be possible to alter a vaccination campaign or other disease management strategy to target a particular subset of the population, particularly in the case of jackals which are often dispersed and cryptic, unlike those in this study; targeting vaccination may be more expensive than trying to treat the population as a whole. But this may not be the case for populations of domestic species such as dogs. It would be more feasible to target a core group of dogs, identified, for example by the owner's social status (i.e. low, middle or high income class) or geographical region.

It is recommended that future studies of disease transmission in wildlife should aim to collect detailed behavioural data on contact rates, despite the associated logistical and financial expenses. There is more scope in wildlife epidemiology studies for further detail on contact behaviour and further studies are required so that cross-

study comparisons of, for example, sex-related differences in contact rates, can be made to see if such trends apply to other jackal populations and species.

Where behavioural observations are not at all possible or further clarification is required alternative methods may be used instead of, or in conjunction with behavioural studies. The jackal population in this study is unusual in that it is highly aggregated and easily observable, characteristics which facilitated the collection of relatively detailed contact data. One approach to elucidating contact patterns within a population would be to 'track' the transmission of a pathogen rather than the movements of the hosts themselves. This approach has been applied to the study of human contact networks for the transmission of tuberculosis by the DNA fingerprinting of *Mycobacterium tuberculosis* isolates (Dillaha *et al.*, 2002, McElroy *et al.*, 2002, Sun *et al.*, 2002, Drobniewski *et al.*, 2003, Rothenberg *et al.*, 2003) and for the investigation of the epidemiology of *M. bovis* in wildlife (De Lisle *et al.*, 2002). Similar technology has been applied to identify wildlife species as sources of *Salmonella* spp. (Refsum *et al.*, 2002, Liebana *et al.*, 2003) and the partial sequencing of CDV isolates from the Serengeti shed further light on the inter-species transmission of this pathogen (Carpenter *et al.*, 1998). It may not be possible to elucidate contacts using the pathogen of interest if infection results in a high mortality rate. It may therefore be more informative to follow a highly transmissible, non-pathogenic and easily genetically typable virus for which the transmission routes and pathogenesis are known.

The analysis of genetic variation in the jackal population itself could also provide an insight into the frequency of emigration and immigration of jackals in the coastal desert belt. Indirect estimates of the number of individuals moving between populations per generation can be generated from measures of genetic differentiation between populations (Wright (1931) cited in Paetkau *et al.* (2004)). Hence microsatellite markers for a wide variety of species have been used in investigations of movements between populations in a variety of contexts including the effects of habitat fragmentation on dispersal (Galbusera *et al.*, 2004, Paetkau *et al.*, 2004). This study collected a large number of tissues samples for the purpose of genetic analysis

and these will hopefully be analysed in the future. It may be that the coastal desert region effectively isolated jackals from populations further inland and that movement inland would only be possible along flooded ephemeral rivers (when they flood sufficiently to reach the coast). But movement between the coastal and inland populations may be much more frequent and this would have important implications for the transmission and persistence of diseases.

In conclusion, this study has provided a number of useful insights into variation in contact rates within a population and shown that it is possible to obtain estimates of disease-specific contact rates. Such insights may prove useful in the design of future behavioural or epidemiological studies in jackals and other wild canid species, and in the interpretation of existing studies and mathematical models of disease persistence. If sufficient behavioural contact data were to be collected from a variety of black-backed jackal populations it may be possible to investigate, using epidemiological models, the relationship between contact rates and population density in this species which would facilitate predictions of disease persistence in different populations.

## **Chapter 7: General Discussion**



This thesis explored the epidemiology of canine pathogens in the Namibian coastal ecosystem, focussing on patterns of infection and epidemic spread in the black-backed jackal, and intra- and inter-specific interactions to understand the dynamics of disease in a multi-host system. Chapters 3 and 4 investigated the spread of a canine distemper epidemic which affected jackals and domestic dogs and the potential exposure of the sympatric seal population to CDV and other morbilliviruses. Chapter 5 presented the results of a serosurvey for a variety of canid pathogens commonly found in domestic dogs and assessed the evidence for maintenance and endemicity within the jackal population. In Chapter 6 jackal-jackal contact rates were analysed to determine if the 20:80 rule applied to the Cape Cross population and whether any one subset of the population made significantly more contacts. Intra- and inter-specific jackal contacts and home range overlap at the colony were used to explain the high prevalence of exposure to CDV attained at the colony during the epidemic.

## **7.1 Jackal 'reservoir'?**

This study found no evidence to support the hypothesis that jackals on the coast may be a maintenance population for CDV. The occurrence of CDV in the jackal population requires introduction from outside the coastal system, which likely occurs via domestic dogs as the sequence of the canine distemper outbreaks suggests that the epidemic on the coast was a result of spill-over from the outbreak in Windhoek's domestic dogs. Domestic dogs on the coast may be (part of) the maintenance population for CDV but this hypothesis was not addressed directly in this study.

Contact between black-backed jackals and domestic dogs on the Namibian coast greatly facilitated the introduction of CDV in this system. The high population density of jackals at the seal colonies and movement between the colonies ensured that CDV 'burned' rapidly through the coastal jackal population, to later spill-back into the domestic dog populations in the south. This highlights the potential for disease transmission between jackals and dogs, and given the levels of exposure to

CAV-1, sarcoptic mange and CHV, CDV is likely not the only pathogen to transmit between these two species.

The maintenance and spread of canid pathogens on the coast will also be facilitated by the growth of the urban domestic dog populations. Of particular concern is the town of Lüderitz because of its proximity to the Diamond Area, which was recently declared a protected area (Dentlinger 2004). Lüderitz recently benefited from the construction of a new industrial and tourist port, and a waterfront development which have attracted new investment and regeneration. But these changes have resulted in a large surge in the influx of migrant workers and rapid growth of the township and shanty areas. These areas are likely to support the majority of the unvaccinated and unrestricted dog population (pers. obs. S. Gowtage) and an increase in this dog population will result in increased contacts with wildlife with more opportunities for disease transmission.

## ***7.2 Control of the canid pathogens and their hosts***

The control of canine distemper and other canid pathogens in the jackal and dog populations may be deemed necessary for the prevention of increased mortality in the seal, brown hyena and IUCN red-list species (e.g. the aardwolf and the bat-eared fox) populations of the coast. Control programmes must target the domestic dog population in order to prevent introduction of disease into the jackal population and these programmes will have to contend with re-infection from jackal and inland domestic dog populations.

A combined approach, of vaccination and dog population management, to the control of distemper would likely be most effective. The removal of stray or unwanted dogs should be combined with increased vaccination coverage. Local Town Councils already round-up stray dogs on a fairly regular basis but this effort could be increased to a monthly basis with a systematic rotation to ensure that all suburbs and industrial areas were covered. Increased vaccination presents more of a challenge as

multivalent vaccines such as Vanguard<sup>®</sup> Plus 5 (Pfizer Animal Health, USA), which protect against canine distemper (as well as CAV-1 and 2, canine parainfluenza virus and CPV-2) are not available free of charge, unlike rabies vaccinations. But it may be possible to reduce the cost of these vaccinations with donations from the larger pharmaceutical companies and with support from the Namibian Government. At the moment the only infrastructure available for the vaccination of domestic dogs, aside from veterinary clinics, is the call to rabies vaccination points after the occurrence of one or more cases. But this could be extended to provide vaccination on a regular basis and this infrastructure used to administer other vaccines, thus reducing the cost of their distribution.

An alternative control strategy would be to reduce contact between domestic dogs and jackals and brown hyenas. But the construction of large tracts of fencing would not only be expensive and unsightly, but prohibited in the large protected areas on the coast. In addition, the design of jackal-proof fencing is no mean feat as jackals are able to pass through game-proof fencing (Barnard 1979) and small livestock farmers in South Africa encountered numerous difficulties (Beinart 1998). Restricting the movement of domestic dogs, however, may be a more feasible alternative with penalties awarded to owners who allow their dogs to roam free and enforcement of legislation which requires dogs to be held in an enclosure and on a leash when off the owner's property.

The culling of the jackal population, in Namibia and elsewhere in southern Africa, is not likely to provide an effective form of disease control, as seen from past experience with other host-pathogen systems. Culling aims to reduce the density of infected and susceptible individuals in a host population, and therefore the probability of transmission between individuals, below the threshold for disease maintenance (Artois *et al.*, 2001). This strategy has been applied to a wide range of wildlife populations including badgers (*Meles meles*) for the control of bovine tuberculosis (Donnelly *et al.*, 2003), wild boars for the control of classical swine fever (Laddomada 2000), domestic dogs for the control of visceral leishmaniasis (Courtenay *et al.*, 2002), and for the control of fox rabies in Europe (Macdonald

1980) with limited success. The reduction in population size has failed for a number of reasons, including lack of knowledge regarding the required level of reduction for the elimination of the disease, the compensatory increase in host reproduction, survival and immigration, and the requirement for populations to be maintained at a very low level in order to achieve control (Artois *et al.*, 2001). The existence of a core group of transmitters in host populations, as indicated by the differences in contact rates (Chapter 6) between jackals in this study, will require extensive coverage of any control measure, including culling, in order to ensure removal of those individuals most responsible for the transmission of the pathogen (Woolhouse *et al.*, 1997) and this is often not logistically and financially possible, or even desirable. The failure of extensive culling in South Africa to control the jackal as an agricultural pest problem provides a further indication that this method is unlikely provide long-term control of the spread of rabies, CDV and other canid pathogens in this species. The South African Government provided financial rewards for the culling of black-backed jackals as a means of controlling the problem of predation on sheep. Over 360,000 jackals were culled in the late 19<sup>th</sup> and early 20<sup>th</sup> centuries and jackals became harder to catch or poison, hence the emergence of a 'super-jackal' (Beinart 1998).

### ***7.3 Future investigations: the Namibian Coast***

This study provided epidemiological evidence for the transmission of canine distemper between dogs and jackals and evidence of natural CDV infection, and of exposure to other canid pathogens, in both these species. But additional investigations are required in order to fully understand the dynamics of canine distemper and other canid pathogens in the coastal system.

Firstly, a longitudinal study should be set-up, monitoring both the domestic dog and jackal populations, with large sample sizes than this study, in order to provide conclusive evidence of the roles played by these species in the transmission and maintenance of CDV and other pathogens. Continued studies must also use an

improved case definition of CD in jackals and dogs which is based upon more than one clinical sign as well as one laboratory test. A longitudinal study would also help assess whether there is continued exposure to CDV in these species and to detect the start of another epidemic, the occurrence of which cannot be ruled out. Continued sampling of the jackal population is currently underway which will help to confirm if CDV did indeed fade-out in this species. Secondly, it will be necessary to isolate CDV RNA from the tissues of both jackals and dogs affected during the 2002-3 epidemic, not only to provide evidence of persistence but to also provide definitive evidence of transmission between these species; this study has collected a large number of tissues for this purpose. The genetic characterisation of CDV isolates can be performed by the amplification, sequencing and phylogenetic analysis of the phosphoprotein (P), nucleocapsid (C), haemoagglutinin (H) or fusion protein (F) gene sequences. Reverse transcription polymerase chain reactions (RT-PCRs), or nested variations of this method, which use primers for one or more of the above listed genes are highly specific and sensitive techniques which have been applied in numerous studies for the comparison of isolates, and the investigation of morbilliviral transmission between species and geographical areas (Barrett *et al.*, 1993, Carpenter *et al.*, 1998, Frolich *et al.*, 2000, Martella *et al.*, 2002, Stanton *et al.*, 2002, Ek-Kommonen *et al.*, 2003, Maes *et al.*, 2003).

Thirdly, it is necessary to obtain further information on the ranging and contact behaviour of jackals further north, south and inland from the seal colonies, and in the urban localities, to assess the frequency and types of encounters which may transmit diseases to domestic dogs and other wildlife. A dog ecology study would greatly contribute to our understanding of the role played by dogs in the transmission of CDV and other pathogens in this system. Such a study would identify those populations which may be maintaining the virus and which are at most risk of another CDV epidemic. A dog ecology study such as that of the dogs in the Zimbabwean communal lands (Butler 1998) would detail the ecology of dogs in on the coast with data on population size, demography, vaccination coverage, contact with wildlife and ranging behaviour, and therefore provide data for improved disease control and population management.

Lastly, an intervention study would provide final confirmation of jackal-dog transmission. If, as proposed in this thesis, jackals cannot maintain CDV endemically and dogs are the original source of the virus, an intervention, such as vaccination, designed to achieve the elimination or effective control of CDV in domestic dog population, should result in the absence or a significant decrease in jackal cases, be it in an epidemic or endemic situation.

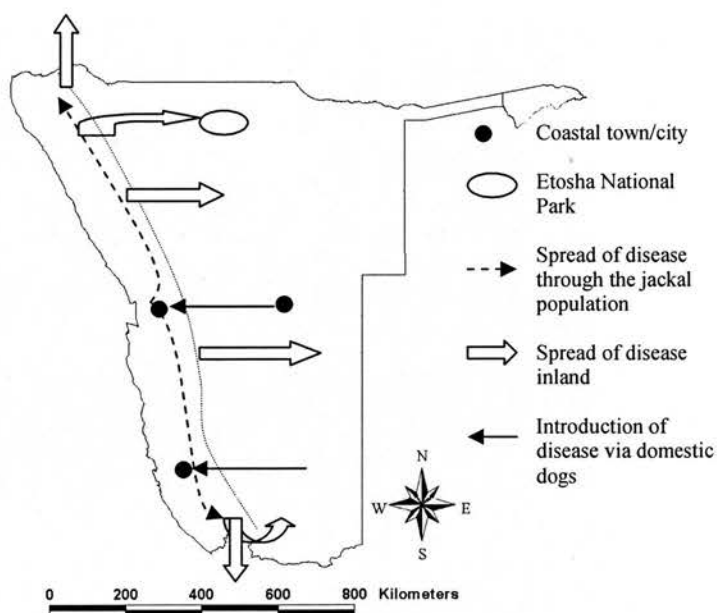
As discussed in Chapter 4, there is still a degree of uncertainty surrounding the exposure of the Namibian Cape fur seal population to morbilliviruses. It would be of considerable value to future studies attempting to assess the risk of spill-over from terrestrial carnivores if the strain of CDV responsible for the outbreaks in jackals and dogs of the coast could be compared to the isolates responsible for the mass mortalities of Caspian and Lake Baikal seals. Genetic analyses may be able to elucidate any differences in receptors which enabled the Caspian/Lake Baikal strains to infect, and be transmitted by, seals. Host-specific differences in innate susceptibility and exposure to co-factors such as immunosuppressive pollutants will also influence the outcome of a CDV infection in a seal. Continued serosurveillance of both the Namibian and South African colonies is advisable as this will help detect the presence of infectious diseases and aid the swift implementation of any control measures (Mörner *et al.*, 2002). A longitudinal (serum) database will also provide a point of reference for the study of other infectious diseases and the role of disease in any future mass-mortalities. Investigations of disease in stranded seals rescued in Namibia and in South Africa may yield valuable insights into pathogens circulating in this seal population.



## 7.4 Jackals as 'vectors': implications for carnivore conservation in Namibia and further a field

The study of the coastal system has shown that the jackal population of this area played a significant role in the transmission of CDV and other generalist canid pathogens and this may have implications for carnivore conservation in other areas of Namibia. The key conclusion is that once CDV had been introduced into the coastal jackal population, the high population density and contact rates allowed CDV to spread very rapidly south along the coast and to (re-)infect new areas. This poses a risk not only to carnivores on the coast but also to species in other areas of Namibia. The coastal desert belt can be regarded as an effective corridor for the rapid spread of CDV and other canid pathogens north and south along the length of Namibia (Figure 7-1). The transmission of disease from coastal to inland jackal populations, or the movement of infected jackals inland, would likely result in the spill-over to inland populations and spread of disease inland, south to South Africa or North into Angola.

**Figure 7-1: The potential spread of disease north and south along the Namibian coast and further inland following spill-over from domestic dogs at urban centres.**





Outside of the coastal system, the area of particular concern with regard to the spread of canine distemper, rabies and other canid pathogens, is the Etosha National Park and the area immediately surrounding the park. The Etosha National Park is, at 2,227,000 km<sup>2</sup>, the largest of the inland nature reserves and with the large diversity of wildlife, it is also the most visited (Baker 1996). This relatively small National Park supports 114 mammal species, 340 bird species, 110 reptile species, 16 amphibian and 1 fish species (Baker 1996). Large felids are well represented in this park with substantial lion (estimated at 350 individuals in 1998 (Briggs *et al.*, 1998)), cheetah, caracal (*Felis caracal*) and leopard populations; spotted and brown hyenas are also found in the park together with cape foxes (*Vulpes chama*) and black-backed jackals (Baker 1996).

Several factors make disease a concern for carnivore conservation in this area. Firstly, contacts between species are increased during the dry season (May to October) during which large numbers of animals congregate at the waterholes along the edge of the salt pan (Baker 1996), thus increasing the opportunities for disease transmission. Secondly, smaller wildlife such as jackals are able to move in and out of the fenced park (Barnard 1979, Briggs *et al.*, 1998). And thirdly, the park lies in the Omusati, Oshana and Oshikoto regions which, together with the Ohangwena region, constitute the NCD. The study by Sorin and Mvula (2001) indicated that this region supports a fairly large and predominantly rural domestic dog population, estimated by Sorin and Mvula to be approximately 115,000 individuals. But this study was based on a limited sample of the population and calculations using updated human census data (Appendices, Chapter 2) estimate the dog population of the NCD at over 180,000 individuals. Rabies vaccination coverage in the rural (10.4-15.3%) and urban (9.7%) areas of the NCD is not sufficient to control rabies and the incidence of human cases has increased since 1997 (Sorin & Mvula 2001). CDV vaccines are not distributed to the general public free of charge or at a reduced cost and are only available through veterinary clinics used by the small fraction of the population which can afford them, hence CDV vaccination coverage is likely to be very low as in other domestic dog populations in South Africa (Leisewitz *et al.*, 2001).

Rabies is also a problem in Etosha with the majority of cases recorded in kudu (*Tragelaphus strepsiceros*), jackals, bat-eared foxes and lions (Berry 1993). Three re-introductions of African wild dogs in Etosha have failed, partly because of rabies (Scheepers & Venzke 1995) which is thought to have been contracted from jackals (Hofmeyr 2001). The potential for the inland jackal populations in Namibia to spread canid disease is illustrated by their involvement in rabies epidemics in diverse areas of the country. Jackals are also thought to have contributed to the large outbreaks of rabies in kudu which affected Etosha and other areas in the late 1970s and early 1980s (Barnard & Hassel 1981) and which still continue to occur (ProMed-mail 2002). Domestic dogs are suspected to be the original source of the rabies virus causing the epidemics in kudu as analyses of kudu rabies virus isolates identified the virus to be a strain commonly found in dogs in many African countries (Hubschle 1988). A time series analysis of rabies cases in jackals and dogs showed that the jackal time-series variable was a significant predictor of the domestic-ruminant and dog time-series variables (Courtin *et al.*, 2000) which indicates that jackals may maintain rabies endemically. Jackals may also be involved in the transmission of anthrax, another high profile problem of the Etosha area (Berry 1993) as over 50% of the faeces recovered from vultures, jackals and hyenas were positive for *Bacillus anthracis* (Lindeque & Turnbull 1994).

Clearly, further investigation is required to determine the risk posed by canid pathogens in domestic dogs and jackals both within and in the areas surrounding the Etosha National Park. As a first step in this direction, a multi-disciplinary carnivore research project, which encompasses the Etosha National Park, the Bushmanland/Caprivi regions in Namibia and the Hluehluwe/Umfolozu Park in South Africa, is conducting extensive serosurveys of lions, spotted hyenas, wild dogs, cheetah, leopard, African wild cat (*Felis lybica*) and black-backed jackals (Briggs *et al.*, 1998). Of 270 lions sampled between 1992 and 1998, 156 were tested for exposure to CDV revealing a seroprevalence of 12.8% (95% CI 8.0-19.1) (Briggs *et al.*, 1998). The potential for transmission of CDV from wildlife in this area is supported by the authors' observation that the majority of the seropositive

individuals were from Etosha; this was contrary to expectations, as the other areas in the study were situated closer to humans with domestic dogs (Briggs *et al.*, 1998).

The past exposure of felids to canine distemper is not *per se* a problem but the virus may evolve to a more pathogenic strain and result in high mortality, as is thought to have occurred in the Serengeti (Roelke-Parker *et al.*, 1996). The 1994 distemper epidemic in the lions of the Serengeti resulted in high morbidity and mortality but lions had been exposed to CDV prior to this outbreak and not suffered any CDV-associated mortality (S. Cleaveland, C. Packer & T. Lembo, unpublished data). A similar scenario occurred in a separate population of lions living in the Ngorongoro Crater which suffered high mortality in 2001 but were exposed to CDV prior to this outbreak (Kissui & Packer 2004). This variability in the pathogenicity of CDV raises concerns about the exposure of the Etosha lion population. Furthermore, the effects of a distemper epidemic in this population may have serious long term consequences as small populations of lions are not thought to be as resilient to CDV as larger populations (Cleaveland *et al.*, 2004). The lion population of the Serengeti has recovered to pre-epidemic levels but that of the Ngorongoro Crater, which suffered 35% mortality in 2001 and repeated disease outbreaks since then, has not yet recovered (Cleaveland *et al.*, 2004, Kissui & Packer 2004).

Finally, the adaptability of the jackal and the resulting variation in social structure and behavioural ecology will mean variation in the role this species plays in the transmission of generalist canid pathogens, ranging from a link in the chain of transmission (i.e. vector) between wildlife and domestic species to an independent maintenance population. Jackals may not act as a maintenance species for CDV in every area in which this disease occurs but their presence will likely ensure the rapid spread over a large geographical area and increase the potential for inter-specific transmission, not only of CDV but also of rabies and other generalist canid pathogens. Carnivore conservation programmes must therefore incorporate location-specific multi-disciplinary studies for the assessment of disease transmission from wild canids such as the jackal and control programmes must be based on thorough investigations of maintenance population(s) in the area of concern.

## **Appendices**

# 8.1 Chapter 2

## 8.1.1 Capture Datasheet

ID label: \_\_\_\_\_ Re-capture: yes / no Method: box trap / foothold / other \_\_\_\_\_

Date: \_\_\_\_\_ Time (seen): \_\_\_\_\_ Location capture (GPS): \_\_\_\_\_

Procedure Filmed: \_\_\_\_\_

**HANDLING** Time sedate: \_\_\_\_\_ Who: \_\_\_\_\_ Drugs/dosage: \_\_\_\_\_

Collar colour/freq: \_\_\_\_\_ Collar length (cm): \_\_\_\_\_

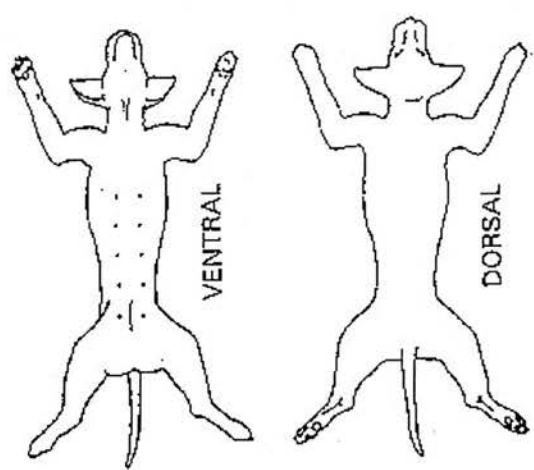
Rototag colour/No.: \_\_\_\_\_ Jumbotag colour/No.: \_\_\_\_\_

Blood sample: \_\_\_\_\_ Blood slides: \_\_\_\_\_ :Ear sample: \_\_\_\_\_ Skin Biopsy: \_\_\_\_\_

Skin Scrape: \_\_\_\_\_ Faecal sample: \_\_\_\_\_ Weight: \_\_\_\_\_ Age class: \_\_\_\_\_

Mange class: 0 none, 1 early, 2 not crusty, 3 crusty, 4 recovering, 5 unknown

Measurements (cm):	cm:
Nose to Shoulder	
Nose to Anus	
Tip Tail bone to base of tail	
Skull length (nose tip to base of skull)	
Shoulder height	
Hind Hock-pad	
Hind Pad	



Photos body/teeth frames: \_\_\_\_\_

Fat reserves (circle): *Thin Lean Optimum Obese Gross* Body Condition(over/underweight): \_\_\_\_\_

**RECOVERY**  
Time Reversal: \_\_\_\_\_ dosage: \_\_\_\_\_

**RELEASE** immediately: yes / no Time: \_\_\_\_\_

**COMMENTS**

8.1.2 Anaesthetics Datasheet

Date: \_\_\_\_\_ Species: \_\_\_\_\_ Sex: \_\_\_\_\_ BW: \_\_\_\_\_

Age: \_\_\_\_\_ Condition: \_\_\_\_\_ Ear tag: \_\_\_\_\_

DRUGS USED (estimated BW 10kg unless otherwise specified):

Name and concentration	Dose	Volume	Route	Time inj.	Real dose received

EFFECT:

Time injected		Time reversed	
Time recumbent		Time first mov.	
Time approached		Time standing	
Time topped up		Time escaped	

MONITORING:

Time											
Heart/min											
Resp/min											
Body temp.											
Eyes (up/down)											
O <sub>2</sub> sat (SpO <sub>2</sub> )											
Reflexes											
Palpebral yes/no											
Pedal yes/no											
Jaw tone (1 to 5)											

INTUBATION: poss \_\_\_\_\_ imposs \_\_\_\_\_ ET size \_\_\_\_\_

NOTES (yes/no and specify time):

Regurgitation: \_\_\_\_\_ Apnoea (breathing): \_\_\_\_\_ Twitching: \_\_\_\_\_

Stiffness: \_\_\_\_\_ Seizures: \_\_\_\_\_

Disrhythmias: \_\_\_\_\_

OTHER OBSERVATIONS: \_\_\_\_\_

### 8.1.3 Post Mortem Datasheet

JACKAL No: \_\_\_\_\_ Collector: \_\_\_\_\_ Date: \_\_\_\_\_  
Cause of death: \_\_\_\_\_ Time (death, approx.): \_\_\_\_\_ Time collected: \_\_\_\_\_

Location (GPS): \_\_\_\_\_

#### CARCASS CONDITION (circle)

**extremely fresh** (as if just died, no bloating)

**slight decomposition** (slight bloating, blood imbibition visible)

**moderate decomposition** (moderate bloating, skin peeling, organs beginning to deteriorate but still recognisable)

**advanced decomposition** (major bloating, skin peeling, organs beyond recognition, bones exposed due to decomposition)

**indeterminate** (no organ tissues present, skeletal remains, may be mummified)

POST-MORTEM Who: \_\_\_\_\_

Blood sample: \_\_\_\_\_ Blood slides: \_\_\_\_\_ Ear sample: \_\_\_\_\_

Skin Biopsy: \_\_\_\_\_ Skin Scrape: \_\_\_\_\_ Faecal sample: \_\_\_\_\_

Weight (kg): \_\_\_\_\_ Age class: \_\_\_\_\_

#### CHECKLIST SAMPLES

STANDARD SAMPLES *In each square, enter:*

✓ = sample taken

Blank = sample not taken or not present

Record any extra samples taken

- ☐ Bladder
- ☐ Spleen
- ☐ Gonadal tissue
- ☐ Lymph nodes: mesenteric, neck, other

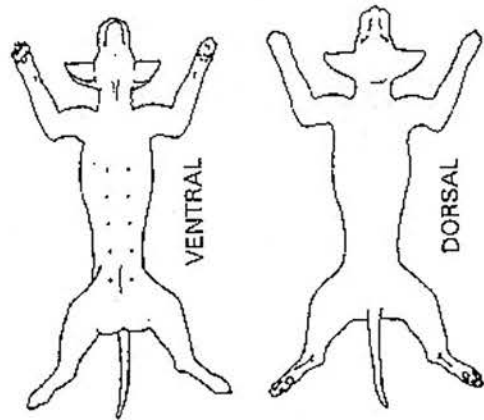
#### b) EXTRA SAMPLES

- ☐ intestine
- ☐ adrenal glands
- ☐ brain
- ☐ bronchial ln.
- ☐ heart
- ☐ kidney
- ☐ liver
- ☐ lung
- ☐ mesenteric ln.
- ☐ ovaries
- ☐ spleen
- ☐ testes



Mange class: 0 none, 1 early, 2 not crusty, 3 crusty, 4 recovering, 5 unknown

<b>Measurements (cm):</b>	<b>cm:</b>
Nose to Shoulder	
Nose to Anus	
Tip Tail bone to base of tail	
Skull length (nose tip to base of skull)	
Shoulder height	
Hind Hock-pad	
Hind Pad	



Photos body/teeth frames: \_\_\_\_\_

Fat reserves (circle): *Thin Lean Optimum Obese Gross*      Body Condition (over/underweight):

**COMMENTS**

(Teeth discolouration, missing, fractures, caries, erosion)

**8.1.4 Calculation of human:dog ratios**

**Table 8-1: The average number of dogs per household for each land type category as detailed in the study by Sorin and Mvula (2001).**

	Urban	Rural, high density	Rural, low density
Number of households surveyed	46	350	176
Number of dogs censused in survey	38	478	256
Average number of dogs per household	0.8	1.4	1.5

**Table 8-2: The calculation of the urban human:dog ratio from human census data and the average number of dogs per home, 2001.** (Sorin & Mvula 2001, 2003).

Urban	Human Census Data (2001)			Sorin and Mvula (2001)	
Region	Human population	Proportion Urban	Average home size	Average no. dogs/home	No. dogs
Oshana	161916	0.31	5.4	0.83	7679
Omusati	228842	0.01	5.9	0.83	320
Ohangwena	228348	0.01	6.3	0.83	299
Osikoto	161007	0.09	5.6	0.83	2138
Total	69256				10436
Human:dog ratio	6.6:1				

**Table 8-3: The calculation of the rural human:dog ratio from human census data and the average number of dogs per home, 2001.** (Sorin & Mvula 2001, 2003).

Rural	Human Census Data (2001)			Sorin and Mvula (2001)	
Region	Human population	Proportion Urban	Average home size	Average no. dogs/home	No. dogs
Oshana	161916	0.69	5.4	1.45	29999
Omusati	228842	0.99	5.9	1.45	55678
Ohangwena	228348	0.99	6.3	1.45	52031
Osikoto	161007	0.91	5.6	1.45	37937
Total	710857				175646
Human:dog ratio	4.0:1				

## 8.2 Chapter 4

### 8.2.1 Basic ecology and biology of the Cape fur seal.

Adapted from Smither's Mammals of Southern Africa (Apps 2000) with additional references (Oosthuizen 1991, Wickens *et al.*, 1991, Wickens *et al.*, 1992, Wickens 1993, Macdonald 2001).

**Habitat** Breeding and non-breeding colonies of variable sizes, on the mainland and on islands offshore, extend from southern Angola to Algoa Bay in South Africa. At the last survey in 1991, there were approximately 25 breeding colonies (19 occurring

on island and 6 on the mainland) and 9 non-breeding colonies (4 on islands and 5 on the mainland). Breeding colonies are distinguished from non-breeding colonies by the production of over 1000 pups per year. It has been proposed that human activities, namely guano mining, forced many seals off the islands onto the mainland, resulting in the extinction of a number of island colonies during the 20<sup>th</sup> century (pers. comm. F. Hugo, Seal Alert, South Africa). The total population is estimated at 1.5 million individuals of which approximately 900,000 occur in Namibia.

**Ranging behaviour** Seals from different colonies share feeding grounds but males, which do not have to return to the colony to suckle pups, range almost twice as far as cows and can be found over 500km from the mainland. A lot of movement takes place between colonies, particularly among young seals. This is not a migratory species but dispersal does occur and a tagged individual was recovered over 1900km from its natal colony.

**Description** Small pups are black, weighing between 5 and 6 kg at birth. Cows are up to 1.6m in total body length and weigh up to 75kg. Bulls are up to 2.1m in length and can weigh up to 190kg. Adults are dark to light brown in colour with males typically darker than the cows.

**Life history** Bulls do not spend all year at the breeding colonies as they haul-out in October, when the cows are offshore feeding and most of the pups have been weaned. The bulls establish and defend territories into which cows are herded to give birth and mate. The majority of cows (90%) give birth in November and December; one pup per cow. Cows mate 6 days after birth but the fertilised egg does not implant immediately. Pups suckle immediately after birth and take solid food at 3-4 months, start swimming at 5-6 months, moving offshore at 7 months. Weaning is very regular, peaking between 8 and 11 months after birth but cows may suckle for as long as a year. Pups and cows identify each other by sniffing and calling and cows will not normally suckle a pup which is not their own. This species typically has a long lifespan with models indicating that 6% of the population exceed 20 years of

age. Cows reach sexual maturity at 3-6 years and males at 4-5 years of age but they do not normally mate until they reach 9-12 years of age.

**Diet** Cape fur seals are one of the top predators in the Benguela system. The majority of the diet consists of small schooling fish but squid, octopuses, crayfish, lobster and other crustaceans are also consumed. Whether or not the seal population causes significant loss to the fishing industry is a hotly debated issue but claims that the fishing industry is supporting an artificially large seal population through loss in catches are considered unfounded.

### **8.2.2 Mass mortality of Cape Fur Seals (*Arctocephalus pusillus pusillus*) in Namibia, 1994.**

Anselmo S, Hart T, Vos H, Groen J, Osterhaus A.

A publication of the Seal Rehabilitation and Research centre, Pieterburen, the Netherlands.

#### **INTRODUCTION**

News of a mass mortality of fur seals in Namibia was received in the Netherlands on June 5<sup>th</sup> 1994. The SRRC (Seal Rehabilitation and Research Centre in Pieterburen) responded immediately in order to help determine the cause of this disaster. Fieldwork to collect samples for virology and toxicology took place on the 9<sup>th</sup> and 13<sup>th</sup> June in Lüderitz, Namibia.

The South African or Cape Fur Seal (*Arctocephalus pusillus pusillus*) is one of the eight known species of *Arctocephalus* in the subfamily *Arctocephalinae* of the *Otariidae*.

The fur seal is the only indigenous breeding pinniped in southern Africa. The total population is estimated to be 1.1 million. This population is distributed mainly along the West coast (93 percent), and 7 percent on the South coast between False Bay and Algoa Bay (Shaughnessy, 1985).

This report focuses on the Namibian population and situation. Fieldwork was undertaken in Wolf and Atlas bays, areas particularly affected. An estimated 800,000 seals are found in Namibian waters, comprising 70 percent of the total population of the species. The largest Namibian colonies are found in Wolf and Atlas bays, which are central for the distribution of the species.

It was estimated that approximately 200,000 pups were born in December 1993, of which 50 percent had died by the time of June. This represents an extremely high percentage if one considers an estimated natural mortality of 25 percent among normal pups in the first 2 to 3 months of their life (Personal communication Dr. J. P. Roux).

By November 1994 the entire group of newborn pups of 1993/1994 had died. Also for 1994/1995 abortions and a higher pup mortality seems to affect the number of pups born in Namibia in a negative way.

Another mass mortality of Cape fur seals on the southwest coast of Africa in 1828 (described by Captain Benjamin Morrell) was reported by Wyatt (1980). A dinoflagellate bloom or 'red tide' was suggested to be the cause of this earlier mortality. When ingested via affected fish the dinoflagellate may be toxic to seals. In the period preceding the mass mortality reduced catches of hake were reported by Dutch fishing vessels operating off the Namibian coast. The fish might have migrated elsewhere.

## DESCRIPTION OF THE SPECIES

*Arctocephalus pusillus pusillus* is the largest of the fur seals: Adult males are about 2.3m nose to tail length and weight 200-350kg depending on the season; females are smaller, being 1.5-1.8m in length and weighing about 120kg (Rand 1956). Pups are born during the end of November and beginning of December.

Pups are about 60-70cm in nose to tail length, and weigh 4.5-7.0kg, with the male being heavier than the female. Pups suckle immediately after birth and continue to do so for nearly a year. The mother remains with her pup for the first week, but then goes to sea for a day or two, gradually increasing the time spent away until the second month, when the pup may be left alone for a period of two weeks. By the time pups are 4<sup>1/2</sup>-5 months old, the males may weight 22kg and females 17kg (Rand 1956). It is not until June or July that the pups are effectively foraging and supplementing their milk diet with crustaceans, cephalopods and small rockfish.

The Cape fur seal is a monoestrous species with a winter mating season followed by a long period of pregnancy. Virgin cows mate after their second winter and may have an estimated gestation period of nearly fifteen months. Mature cows go into heat about six days after pupping (Rand 1956).

## FINDINGS

Fieldwork by SRRC personnel was carried out between June 9-13 at different colonies: Hottentot, Atlas and Wolf bays.

Rookeries were covered with dead and moribund pup bodies (pups aged app. 6-7 months).

The majority of pups still alive were starved, lethargic and dehydrated. No signs of any specific disease were apparent. The entire picture suggested a problem of starvation. Very few subadults and adults were present on the rookeries, and those present showed no particular interest in pups.

During our stay, we observed only few emaciated females nursing up to three pups.

A total of 41 dead seals were dissected. These consisted of 40 seal pups about 6-7 months old and 1 subadult.

Necropsies were performed in all the three colonies previously mentioned: 2 seals at Hottentot Bay, 23 seals at Atlas Bay and 16 at Wolf Bay. All seals were estimated dead less than 24 hours, except for the subadult male.

Blood samples were taken from 15 live pups during the final two days of fieldwork.

## NECROPSY REPORT

### **External Examination:**

All animals were emaciated.

Of the 40 pups dissected, 22 were male with an average weight of 7.2kg and average length 82.9cm; 18 were females, with an average weight of 6.2kg and average length of 77.1cm.

### **Internal Examination:**

#### *Blubber*

Blubber was present in only two seals which were killed by a brown hyena, the thickness of this being respectively 21 and 17mm. All the other animals had not detectable blubber layer.

#### *Abdominal cavity*

#### LIVER

- Change in colour (yellow) – degeneration – observed in 14 seals.
- Dark areas in both liver lobes, with very clear limits – observed in 3 seals.
- Superficial presence of small red spots 1-2mm diameter – observed in 2 seals.
- Epithelial area of erosion about 4 mm diameter covered by bubbles – observed in 1 seal.

#### STOMACH

- Empty – observed in 40 seals.
- Presence of stones – observed in 3 seals.
- Gastric dilation – observed in 1 seal.
- Hemorrhagic gastritis – observed in 20 seals.
- Full of Rock Lobster – observed in 1 seal.

#### INTESTINE

- Hemorrhagic enteritis – observed in 38 seals.
- Large intestine with white spots of 2mm on the wall, corresponding to ulcers in the mucosa – observed in 1 seal.



### *Thoracic cavity*

All the seals had several small areas of emphysema in the lungs. No other special remarks.

## VIROLOGICAL STUDIES

During necropsies, samples were taken for virological studies. These samples included serum samples and organ materials. Organ materials always included tissue samples from lungs, liver, kidneys, spleen, heart, brain. From eight animals additional samples were taken from the small and large intestine and from the mesenterial and intestinal lymph nodes. Tissue samples were stored at 4°C, and transported to Erasmus University Rotterdam, The Netherlands, within 48 after sampling.

## SEROLOGY

Serum samples taken from 23 animals were tested for the presence of antibodies against morbilliviruses (canine distemper virus (CDV) IgG ELISA), phocine herpes virus-1 (PHV-1, virus neutralization), canine adenovirus (IgG and IgM ELISA), canine parvovirus (IgG and IgM ELISA), canine coronavirus (IgG and IgM ELISA), and canine parainfluenza viruses type 1 and type 2 (IgG and IgM ELISAs). Detectable serum antibody titers were only found against CDV and canine adenovirus (IgG ELISA). All other titers were below the detection limit of 1:50.

### *Morbillivirus-specific serum antibodies*

Using the CDV IgG ELISA, morbillivirus-specific serum antibodies were detected in 15 of 23 animals tested (65%, table 1: titres equal to or higher than 30 are considered positive). To obtain more information about the nature of the morbillivirus that had induced these titres, the sera were subsequently tested in virus neutralization assays against both CDV and phocine distemper virus (PDV). Results of these assays showed the presence of both sera with higher PDV titres as well as sera with higher CDV titers (figure 2). On the basis of these data it is hard to conclude whether only one or two different morbilliviruses have infected this population in the past. Furthermore, the age of the pups does not fully allow to rule out the possibility that serum antibodies found are still of maternal origin. During necropsies no indications were found of possible morbillivirus infection (see above). In addition, no morbillivirus could be isolated from tissue samples of these animals (see below). We therefore conclude that although a morbillivirus is enzootic in the population of Cape fur seals, it probably did not play a direct role in the mass mortality.

### *Canine adenovirus*

IgG but no IgM ELISA titres against canine adenovirus were detected in all sera tested (table 1). Although it is likely that an adenovirus is indeed enzootic in this population, no adenovirus was isolated in a series of cell cultures (see below). In addition, no adenovirus antigen could be demonstrated in the livers of deceased animals using fluorescence techniques, which suggest that an adenovirus was not the primary cause of the mass mortality.



## VIRUS ISOLATION STUDIES

Organ materials were processed following standard procedures. Organ homogenates were co-incubated with Vero cells (monkey kidney cell line), CRFK cells (feline kidney cell line) or seal kidney cells (from harbour seal origin). Cell cultures were routinely screened for cytopathic changes during a period of several weeks. No viruses could be isolated.

## PRELIMINARY CONCLUSIONS

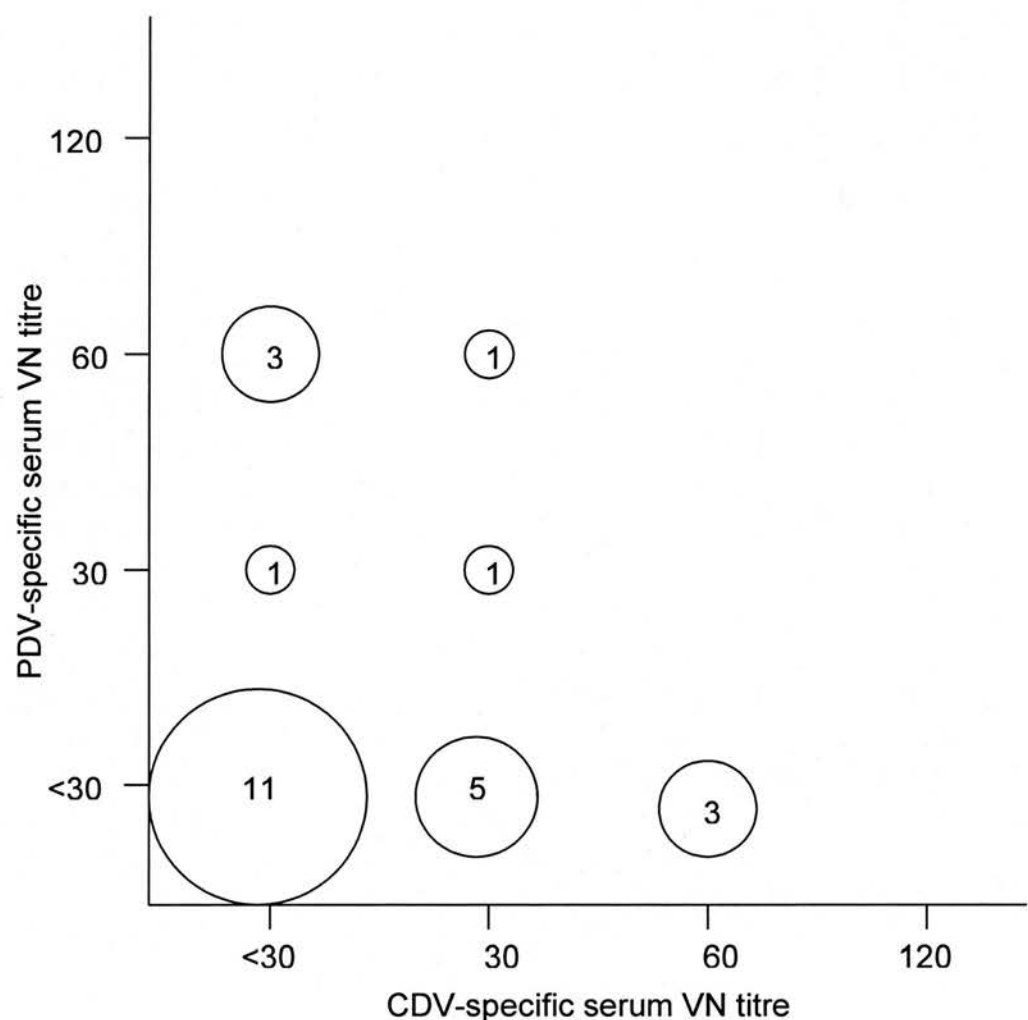
The mass mortality among Cape Fur seals primarily affected pups. The general picture indicated that the pups suffered from chronic starvation. Of particular interest is the fact that dissected pups aged 6-7 months had body weights in the same range as those reported for newborns of the same species by Rand (1965). No indications were found for a role of an infectious disease in the mortality.

This hypothesis fits with the fact that few subadult and adult animals were present in the colony: these animals probably migrated to open waters in search of food.

Table 1: virus specific serum antibody titres in Namibian Cape fur seals

serum no.	CDV IgG ELISA titre	PHV-1 VN titre	canine adenovirus IgG ELISA titre
14	10	<20	136
16	30	nt	105
17	100	nt	109
19	30	<20	103
20	30	<20	142
21	30	<20	91
22	10	<20	125
23	30	<20	154
24	30	<20	148
25	<10	<20	136
27	30	<20	182
29	30	<20	151
30	30	<20	120
32	30	<20	106
33	10	<20	129
34	<10	<20	103
35	100	<20	123
36	30	<20	104
37	<10	<20	113
38	<10	<20	95
40	100	<20	102
44	<10	<20	337
45	100	<20	137

Figure 2



25 sera were tested in a virus neutralization assay against phocine distemper virus (PDV) or canine distemper virus (CDV). The titers are plotted against each other; titers equal or higher than 30 are considered positive. The numbers in the graph represent the number of sera with the indicated titers: e.g. 3 animals had a CDV titer <30 and a PDV titer of 60.

## 8.3 Chapter 5

### 8.3.1 Manuscript, Faecal sample analysis

**Prevalence of parasitic ova and coccidian oocysts in free-ranging black-backed jackals (*Canis mesomelas*) from Namibia.**

C.D. Pollard<sup>\*x</sup>, M.T. Fox<sup>\*</sup>, S. Gowtage-Sequeira<sup>#</sup>, S. Funk.

<sup>\*</sup>The Royal Veterinary College, Royal College Street, London NW1 0TU, UK; <sup>#</sup>The Centre for Tropical Veterinary Medicine, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Roslin, Midlothian EH25 9RG, UK. <sup>x</sup>Corresponding author.

#### ABSTRACT

Faecal samples from 35 free-ranging black-backed jackals captured in Namibia were examined by direct microscopy. The age range of the animals was from less than six months to over three years. Faecal egg counts were estimated during direct microscopy examinations. Parasite eggs and oocysts, comprising the phyla Nematelminthes, Acanthocephala, Platyhelminthes and Protozoa, were identified. The overall prevalence of parasitism in the samples was 71.4% (25/35, 95% CI 53.7-85.4). The prevalence of nematode eggs, Diphyllbothrium eggs, Taenid eggs, Acanthocephalan eggs, coccidian oocysts and unidentified eggs was 11.4% (4/35, 95% CI 3.2-26.7), 45.7% (16/35, 95% CI 28.8-63.4), 5.7% (2/35, 95% CI 0.7-19.2), 11.4% (4/35, 95% CI 3.2-26.7), 11.4% (4/35, 95% CI 3.2-26.7), and 14.3% (5/35, 95% CI 4.8-30.3) respectively. One of the nematode eggs was identified as *Toxascaris leonina* (2.9%). The black-backed jackal is a common carnivore in Namibia, notable for its opportunistic and adaptable feeding behaviour. In the study area the majority of its diet would be derived from the marine environment, including scavenging carcasses of the white-breasted cormorant (*Phalacrocorax carbo*) and the Cape fur seal (*Arctocephalus pusillus*). This may account for the high prevalence of *Diphyllbothrium*, a cestode genus found in pinnipeds and marine fish.

KEYWORDS: Jackal; *Canis mesomelas*; *Dipyllobothrium*; Acanthocephala; Cape Cross Seal Reserve.

## INTRODUCTION

The black-backed jackal (*Canis mesomelas*) is a common and widespread African carnivore. The southern distribution of this species encompasses south west Angola, Zimbabwe and southern Mozambique and extends through to the South African coast (Alderton 1994). The Cape Cross Seal Reserve in Namibia is centred on a colony of the Cape fur seal (*Arctocephalus pusillus*), and is located in an area of hyper-arid desert on Namibia's Skeleton Coast. Black-backed jackals are omnivorous and are highly opportunistic scavengers, as well as being capable of co-operative hunting (Sheldon 1992). With the jackals in this study, food of marine origin, in particular the Cape fur seal, is predominant throughout the year (Nel et al 1997).

Black-backed jackals are susceptible to a spectrum of infectious and parasitic diseases similar to that of the domestic dog (Kennedy-Stoskopf 2003). This includes: rabies (Cumming 1982); canine parvovirus, canine distemper virus (Alexander et al 1994); canine adenovirus type 1 (Spencer et al 1999); *Ehrlichia canis* (Neitz and Thomas 1938); and *Ancylostoma caninum* and *Dipylidium caninum* (Round 1968).

## MATERIALS AND METHODS

### *Animals*

The faecal samples for this study ( $n=35$ ) came from captured individuals or were fresh samples collected in the field, between October 2002 and January 2003, as part of a larger research project, the Namibia Jackal Project (NJP). The aims of that project are to study the transmission of infectious diseases of canids, including canine distemper, between carnivores on the Namibian coast. There are two geographically distinct populations; the animals in this study were from the Cape Cross Seal Reserve (CCSR) and the Diamond Area seal colony further to the south.

The majority of the animals (28/35) were from the CCSR, the remainder (7/35) were from the Diamond Area. Seven jackals were examined *post mortem*.

### *Samples*

The exact weight of each faecal sample was unknown, but was within a range of 2-4g. All samples were presented fixed in variable volumes of 10% formalin, in universal containers. For the purposes of this study it was assumed that each sample contained 3g of faeces, in order to facilitate semi-quantitative analysis. The volume of each sample was standardised to 15ml, either by the addition of formalin or, after an hour of settling, by the removal of excess. All samples were sieved to remove large particulate matter.

### *Modified McMaster Flotation*

Quantitative analysis of faecal egg output using the modified McMaster flotation method is usually performed on fresh faecal samples. To ensure that this method would work on the jackal faecal samples, four different flotation solutions (sodium chloride, sucrose, magnesium sulphate and zinc sulphate) were tested using a fixed dog faecal sample. This sample contained high numbers of previously identified parasitic ova. The zinc sulphate solution was selected on the basis of the results of this examination.

### *Direct microscopy*

To allow for semi-quantitative analysis, each sample was mixed thoroughly, and 5ml was removed and allowed to settle for an hour. 4ml of formalin were then removed, leaving roughly 1g (in 1ml) of the original faecal sample. 0.1ml of this was examined by direct microscopy, all parasitic ova were counted, measured and described; this procedure was repeated 5 times for each sample.

## RESULTS

Parasitic ova were found in 25 samples, thus the overall prevalence of parasitism in these jackals was 71.4% (95% CI 53.7-85.4). The prevalence of the different parasitic taxa is given in Table 1.

**Table 1 Prevalence of parasitic taxa**

Taxa	<i>n</i>	Prevalence
Nematoda	4	11.4% 95% CI 3.2-26.7
Cestoda	17	48.6% 95% CI 31.4-66.0
Acanthocephalan	5	14.3% 95% CI 4.8-30.3
Protozoa	4	11.4% 95% CI 3.2-26.7
Unknown	5	14.3% 95% CI 4.8-30.3

Of the four samples that contained nematode eggs, one was identified as *Toxascaris leonina* (2.9%), one as a strongyle egg (2.9%); the other two contained larvae (5.7%). The most prevalent cestode, and the most prevalent parasite egg overall, was *Diphyllbothrium* (Fig. 1) with 45.7% (16/35); the remaining cestode eggs were Taenid, 5.75% (2/35). The protozoa (Fig. 3) were from the genus *Isospora*, possibly either *I. ohioensis* or *I. burrowsi*, based on the dimensions of the oocysts. The range of dimension for all eggs and oocysts is given in Table 2.

**Figure 1 *Diphyllbothrium* egg**



**Figure 2 *Acanthocephalan* egg**





**Figure 3 *Isospora* oocyst**



**Table 2**

Taxa	Size range (µm)
<i>Toxascaris leonina</i>	70x70
Strongyle	61.6x47.6
Other nematode	72.8x70 - 100.8x78.4
<i>Diphyllbothrium</i>	50.4x42 – 56x42
Taenid	33.6x25.2 – 33.6x33.6
Acanthocephalan	81.2x50.4 – 92.4x58.8
<i>Isospora</i>	19.6x19.6 – 28x25.2
Unknown #1	58.8x56 – 61.6x44.8
Unknown #2	165.2x58.8-176.4x84
Unknown #3	75.6x64.4

**Table 3 Frequency, mean EPG, range and Standard Deviation for the different parasite taxa.**

Parasite taxon	Frequency	Mean EPG	Range	S.D.
<i>T.leonina</i>	1	2	/	/
Strongyle	1	2	/	/
Other nematode	2	4	4-8	2.8
<i>Diphyllbothrium</i>	16	207.5	2-2088	525.7
Taenid	2	42	2-82	56.6
Acanthocephalan	4	20.8	2-58	25.2
<i>Isospora</i>	4	15	2-54	26
Unknown (#1,2&3)	5	3.2	2-6	1.8

There was a large variation in the faecal egg output, measured in eggs per gram (EPG), between individual jackals and also parasite taxa (Table 3). Additionally, adult tapeworms were recovered on *post mortem* examination, from the small intestines of sample numbers 14 and 18. These two samples were both positive for *Diphyllbothrium* eggs; however the adult worms have not yet been fully identified. A full listing of results according to sample number is given in Table 1 of the



appendix. 17 of the samples were positive for only one type of parasite, 7 had two types and one had four types (sample 13).

## DISCUSSION

The overall prevalence of parasitism in this study was found to be 71.4%, slightly less than the prevalence of *Ancylostoma* (76%), the most prevalent parasite found in a study on golden jackals *Canis aureus* by Shamir et al (2001). The prevalence of Taenid eggs in this study (5.7%) is similar to that for *Dipylidium caninum* in golden jackals, (5.9%), also from the latter study.

The nematode parasite *Toxascaris leonina* can be found in the small intestine of wild *Canidae*, as well as in domestic dogs and cats, worldwide (Soulsby 1986). It has also been found in golden jackals (Round 1968). The prevalence in this study (2.9%) is much lower than that found in a 10 year study of stray dogs in Belgium (10.1%) by Vanparijs et al (1991).

The acanthocephalan *Oncicola canis* has been found in both wild and domestic canids in North and South America (Soulsby 1986). Acanthocephalans are reported in all vertebrate classes, but are especially common in birds and fish (Smyth 1994); this may be the first report of an acanthocephalan in a black-backed jackal (Fig. 2). Acanthocephalans have also been reported in otariids, including the genera *Corynysoma*, *Bulbosoma* and *Acanthocheilonema* (Gage 2003). The presence of acanthocephalan eggs in these faecal samples could indicate either natural infection or the passage of eggs acquired from prey animals.

In the study area the vast majority of the black-backed jackal's diet was reported to be composed of the Cape fur seal (Nel et al., 1997). Food derived from the marine environment predominates all year round. The consumption of fish, cormorants (Avery et al., 1987) or fur seals could account for the presence of acanthocephalans in these jackals.

Coccidian parasites of the genus *Isospora* have been found in other wild canids, including coyotes *Canis latrans* in New York State (Gompper et al 2003). The prevalence of *Isospora* in this study (11.4%) is much higher than the value obtained in the latter study for *I. ohioensis* (4.5%), *I. heydorni* (3.4%) and *I. canis* (1.1%). The actual prevalence may have been even higher, since it has been demonstrated by Dauschies et al (2001) that autofluorescence microscopy is a better method of detecting *Isospora* oocysts than bright field microscopy, albeit *I. suis* in swine faeces. Furthermore, the time of day when faecal samples are collected can influence the prevalence of coccidian oocysts. In a study of three species of African antelope the prevalence of coccidian oocysts was significantly greater in samples collected in the afternoon (Ezenwa 2003).

The prevalence and mean EPG for *Diphyllbothrium* were by far the largest of any of the parasites recorded in this study. The largest individual EPG value, 2088, was for *Diphyllbothrium*, from sample 22. Diphyllbothrid eggs are discharged continuously, independent of the detachment of segments (Bowman et al 2003). This may account for the large numbers encountered. Many texts report *Diphyllbothrium* as a potentially zoonotic cestode parasite mainly of freshwater, but also of anadromous fish, and the birds and mammals that feed upon them (Urquhart et al 1995; Soulsby 1986; Smyth 1994). However, *Diphyllbothrium* is included in a description of the parasites of domestic dogs by Bowman et al (2003), and has been found in the side-striped jackal *Canis adustus* (Round 1968).

The eggs of *Diphyllbothrium* are operculate and can resemble those of other cestodes and trematodes (Soulsby 1986). *Diphyllbothrium* eggs measuring  $55.63 \pm 2.82 \times 39.42 \pm 5.64$  microns have been reported by Ferreira et al (1984). The eggs described as *Diphyllbothrium* in this study were operculate (Fig. 1) and ranged in size from  $50.4 \times 42$  microns to  $56 \times 42$  microns, and so are consistent with the latter report. *D. lanceolatum*, *D. pacificum* and *D. terapterus* have all been found in pinnipeds (Gage 2003). The normal cycle of *D. pacificum* was reported by Baer (1969) as being between sea lions and marine fish; sea lions are the definitive host.

The high prevalence of *Diphyllbothrium* (45.7%) in black-backed jackals from the CCSR and Diamond Area in this study may suggest that this is a natural infection, highlighting a new natural cycle between marine fish and jackals. However, it is also quite likely due to the consumption of Cape fur seal remains containing this parasite, and likewise other marine derived food, in these coastal environments.

Regarding the semi-quantitative aspect of this study, there were two main limitations to the accuracy of the results and sensitivity of methods used to obtain them. Firstly, the main limiting factor was the assumption that all samples contained 3g of faeces. From visual inspection alone it was possible to determine that some samples were of much higher quality than others. Secondly, the presence of lots of particulate matter, usually sand in some samples, decreased the sensitivity of egg counts from direct microscopy.

## CONCLUSION

This study has potentially identified three previously unrecorded parasites of black-backed jackals, namely an acanthocephalan, *Diphyllbothrium* and *Isospora*. The former two could potentially be derived from the scavenging of marine derived food that constitutes the bulk of the black-backed jackal's diet in coastal Namibia. Further investigation into the identity and quantitative aspects of these parasites is warranted.

## REFERENCES

- Alderton, D. (Ed.) 1994 Foxes, Wolves and Wild Dogs of the World. Blandford, pp. 60-142
- Alexander, K.A., Kat, P.W., Wayne, R.K., Fuller, T.K. 1994 Serologic survey of selected canine pathogens among free ranging jackals in Kenya. J. Wildl. Dis. **30**, 486-491
- Baer, J.G. 1969 *Diphyllbothrium pacificum*, a tapeworm of sea lions endemic along the coastal are of Peru. J. Fish. Res. **26**, 717-723
- Bowman, D.D., Lynn, R.C., Eberhard, M.L. (Eds.) 2003 Georgis' Parasitology for Veterinarians. 8th Edition. Saunders, p. 307
- Cumming, D.H.M. 1982 A case history of the spread of rabies in an African country. S. Afr. J. Sci. **78**, 443-447
- Dauguschies, A., Bialek, R., Joachim, A., Mundt, H.C. 2001 Autofluorescence microscopy for the detection of nematode eggs and protozoa, in particular *Isospora suis*, in swine faeces. Parasitol Res. **87**, 409-12

- Ezenwa, V.O. 2003 The effects of time of day on the prevalence of coccidian oocysts in antelope faecal samples. *Afr. J. Eco.* **41**, 192-193
- Ferreira L.F., Araújo, A., Confalonieri, U., Nunez, L. 1984 The finding of eggs of *Diphyllbothrium* in human coprolites (4,100-1,950 B.C.) from northern Chile. *Mem. Inst. Oswaldo Cruz* **79**, 175-180.
- Gage, L.J. 2003 Pinnipedia. In: Fowler, M.E., Miller, R.E. (Eds), *Zoo and Wild Animal Medicine*. Fifth Edition. Saunders, pp. 466-467
- Gompper, M.E., Goodman, R.M., Kays, R.W., Ray, J.C., Fiorello, C.V. 2003 A survey of the Parasites of Coyotes (*Canis latrans*) in New York based on Fecal Analysis. *J. Wildl. Dis.* **39**, 712-717
- Kennedy-Stoskopf, S. 2003 Canidae. In: Fowler, M.E., Miller, R.E. (Eds.), *Zoo and Wild Animal Medicine*. Fifth Edition. Saunders, pp. 482-491
- Neitz, W.O., Thomas, A.D. 1938 Rickettsiosis in the dog. *J. S. Afr. Vet. Med. Assoc.* **9**, 166-174
- Nel, J.A.J., Loutit, R., Bothma, J. du P. 1997 Prey use by black-backed jackals along a desert coast. *S. Afr. J. Wildl. Res.* **27**, 100-104
- Round, M.C. (Ed.) 1968 Check List of the Helminth Parasites of African Mammals. Commonwealth Agricultural Bureaux, p. 131
- Shamir, M., Yakobson, B., Baneth, G., King, R., Dar-Verker, S., Markovics, A., Aroch, I. 2001 Antibodies to Selected Canine Pathogens and Infestation with Intestinal Helminths in Golden Jackals (*Canis aureus*) in Israel. *Vet. J.* **162**, 66-72
- Sheldon, J.W. (Ed.) 1992 Wild Dogs. The natural History of the Nondomestic Canidae. Academic Press, pp. 46-50
- Smyth, J.D. (Ed.) 1994 Animal Parasitology. Third Edition. Cambridge University Press, pp. 302-457
- Soulsby, E.J.L. (Ed.) 1986 Helminths, Arthropods and Protozoa of Domesticated Animals. 7<sup>th</sup> Edition. Baillière Tindall, pp. 129-626
- Spencer, J.A., Bingham, J., Heath, R., Richards, B. 1999 Presence of antibodies to canine distemper virus, canine parvovirus and canine adenovirus type 1 in free ranging jackals (*Canis adustus* and *Canis mesomelas*) in Zimbabwe. *Onderstepoort J. Vet. Res.* **66**, 251-253
- Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W. (Eds.) 1995 *Veterinary Parasitology*. Longman Scientific and Technical, p. 133-134
- Vanparijs, O., Hermans, L., van der Flaes, L. 1991 Helminth and protozoan parasites in dogs and cats in Belgium. *Vet. Parasitol* **38**, 67-73.

## APPENDIX

**Table 1 Distribution of egg/oocysts counts (EPG=eggs per gram)**

Sample No.	EPG N	EPG A	EPG D	EPG C	EPG P	EPG O
1	0	0	36	0	0	0
2	8	0	0	0	0	0
3	0	0	0	0	2	0
4	0	0	0	0	0	0
5	0	0	0	0	0	4
6	0	0	0	0	54	0
7	0	0	0	2	0	6
8	0	2	0	0	0	0
9	0	2	0	0	0	0
10	4	0	36	0	0	0
11	0	0	0	0	0	0
12	0	0	22	0	0	0
13	2	0	2	0	2	2
14	0	0	132	0	0	2
15	0	0	0	0	0	0
16	0	0	274	0	0	0
17	0	0	8	0	2	0
18	0	58	48	0	0	0
19	0	0	0	0	0	0
20	0	0	116	0	0	0
21	0	0	24	0	0	0
22	0	0	2088	0	0	0
23	0	0	172	0	0	0
24	0	0	6	0	0	0
25	0	6	0	82	0	0
26	0	0	0	0	0	0
27	0	36	0	0	0	0
28	0	0	108	0	0	2
29	0	0	40	0	0	0
30	2	0	0	0	0	0
31	0	0	0	0	0	0
32	0	0	0	0	0	0
33	0	0	0	0	0	0
34	0	0	0	0	0	0
35	0	0	0	0	0	0

KEY: N= nematode, P= *Isospora*, A= acanthocephalan, O= unidentified, D= *Diphyllobothrium*, C= other cestode.

## References

- Admasu E, Thirgood SJ, Bekele A, Laurenson MK. 2004. Spatial ecology of golden jackal in farmland in the Ethiopian Highlands. *African Journal of Ecology* 42: 144-52.
- Aguilar A, Borrell A. 1994. Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990-1992 Mediterranean epizootic. *Science of the Total Environment* 154: 237-47.
- Alexander K, Appel M. 1994. African wild dogs (*Lycaon pictus*) endangered by a canine distemper epizootic among domestic dogs near the Masai-Mara National Reserve, Kenya. *Journal of Wildlife Diseases* 30: 481-5.
- Alexander K, Conrad P, Gardner L, Parish C, Appel M *et al.*,. 1993a. Serologic survey for selected microbial pathogens in African wild dogs (*Lycaon pictus*) and sympatric domestic dogs (*Canis familiaris*) in Masai Mara, Kenya. *Journal of Zoo and Wildlife Medicine* 24: 140-4.
- Alexander K, Kat P, Frank L, Holekamp K, Smale L *et al.*,. 1995. Evidence of canine distemper virus infection among free-ranging spotted hyenas (*Crocuta crocuta*) in the Masai Mara, Kenya. *Journal of Zoo and Wildlife Medicine* 26: 201-6.
- Alexander K, Kat P, Wayne R, Fuller T. 1994. Serological survey of selected canine pathogens among free-ranging jackals in Kenya. *Journal of Wildlife Diseases* 30: 486-91.
- Alexander KA, Kat PW, Munson L, Kalake A, Appel M. 1996. Canine distemper-related mortality among wild dogs (*Lycaon pictus*) in Chobe National Park, Botswana. *Journal of Zoo and Wildlife Medicine* 27: 426-7.
- Alexander KA, Smith J, Macharia M, King AA. 1993b. Rabies in the Masai Mara, Kenya: preliminary report. *Onderstepoort Journal of Veterinary Research* 60: 411-4.
- Anderson C, Haroldson M. 1996. *Effectiveness of attractants to lure grizzly bears into hair collection sites for future DNA fingerprinting*. <http://nrmssc.usgs.gov/>. US Geological Survey. Accessed: 2002.
- Anderson R. 1991. Populations and infectious diseases: ecology or epidemiology? The Eighth Tansley Lecture. *Journal of Animal Ecology* 60: 1-50.
- Anderson R, Jackson H, May R, Smith A. 1981. Population dynamics of fox rabies in Europe. *Nature* 289: 765-71.
- Anderson R, May R. 1982. Directly transmitted infectious diseases: Control by vaccination. *Science* 215: 1053-60.
- Anderson R, May R. 1985. Age-related changes in the rate of disease transmission: implications for the design of vaccination programmes. *Journal of Hygiene, Cambridge* 94: 365-436.
- Anderson R, May R. 1991. *Infectious diseases of humans: dynamics and control*. Oxford: Oxford University Press. 757 pp.
- Anselmo S, Hart T, Vos H, Groen J, Osterhaus A. 1995. *Mass mortality of cape fur seals (A.p.p.) in Namibia, 1994*. Report. Seal rehabilitation and research centre, Pieterburen, Netherlands.
- Appel M. 1969. Pathogenesis of canine distemper. *American Journal of Veterinary Research* 30: 1167-82.
- Appel M. 1970. Distemper pathogenesis in dogs. *Journal of the American Veterinary Medical Association* 156: 1681-4.



- Appel M. 1987a. Canine adenovirus Type 1 (Infectious canine hepatitis virus). In *Virus Infections of Carnivores*, ed. M Appel, pp. 29-43. Amsterdam: Elsevier Science Publishers.
- Appel M. 1987b. Canine distemper virus. In *Virus Infections of Carnivores*, ed. M Appel, pp. 133-60. Amsterdam: Elsevier Science Publishers.
- Appel M, Parrish C. 1987. Canine parvovirus Type 2. In *Virus Infections of Carnivores*, ed. M Appel, pp. 69-92. Amsterdam: Elsevier Science Publishers.
- Appel M, Summers B. 1995. Pathogenicity of morbilliviruses for terrestrial carnivores. *Veterinary Microbiology* 44: 187-91.
- Appel MJ, Yates RA, Foley G, Bernstein J, Santinelli S *et al.*, 1994. Canine distemper epizootic in lions, tigers and leopards in North America. *Journal of Diagnostic Veterinary Investigation* 6: 277-88.
- Apps P, ed. 2000. *Smither's mammals of Southern Africa: A field guide*. Cape Town: Struik
- Arjo WM, Gese EM, Bromley C, Kozlowski A, Williams ES. 2003. Serologic survey for diseases in free-ranging coyotes (*Canis latrans*) from two ecologically distinct areas of Utah. *Journal of Wildlife Diseases* 39: 449-55.
- Arlian L. 1989. Biology, host relations and epidemiology of *Sarcoptes scabiei*. *Annual Review of Entomology* 34: 139-61.
- Arlian L, Morgan M, Arends J. 1996a. Cross-reactivity among various strains of *Sarcoptes scabiei*. *Journal of Parasitology* 82: 66-72.
- Arlian L, Morgan M, Rapp C, Vyszenski-Moher D. 1996b. The development of protective immunity in canine scabies. *Veterinary Parasitology* 62: 133-42.
- Arlian L, Morgan M, Vyszenski-Moher D, Stemmer B. 1994. *Sarcoptes scabiei*: The circulating antibody response and induced immunity to scabies. *Experimental parasitology* 78: 37-50.
- Arlian L, Vyszenski-Moher D. 1988a. Energetic relationship of *Sarcoptes scabiei* var. *canis* (Acari: Sarcoptidae) with the laboratory rabbit. *Journal of Medical Entomology* 25: 64-8.
- Arlian L, Vyszenski-Moher D. 1988b. Life cycle of *Sarcoptes scabiei* var. *canis*. *Journal of Parasitology* 74: 427-30.
- Artois M, Delahay R, Guberti V, Cheeseman C. 2001. Control of infectious diseases of wildlife in Europe. *Veterinary Journal* 162: 141-52.
- Arya SC. 2000. Human contact with cattle, dogs and monkeys and genetic diversity of measles viruses. *Comparative Immunology, Microbiology and Infectious Diseases*. 23: 125-7.
- Atkinson RPD, Macdonald DW, Kamizola R. 2002. Dietary opportunism in side-striped jackals *Canis adustus* Sundevall. *Journal of Zoology* 257: 129-39.
- Bailey N. 1975. *The mathematical theory of infectious diseases and its applications*. London: Griffin. 413 pp.
- Baker J. 1992. The pathology of phocine distemper. *The Science of the Total Environment* 115: 1-7.
- Baker J, Ross H. 1992. The role of bacteria in phocine distemper. *The Science of the Total Environment* 115: 9-14.

- Baker L. 1996. An overview of Namibia's gameparks and recreation Areas. In *Namibia Environment*, ed. P Tarr, pp. 32-49. Windhoek: Ministry of Environment and Tourism.
- Barker I, Parrish C. 2001. Parvovirus infections. In *Infectious diseases of wild mammals*, ed. E Williams, I Barker, pp. 131-46. London: Manson Publishing Ltd.
- Barnard BJ. 1979. The role played by wildlife in the epizootiology of rabies in South Africa and South-West Africa. *Onderstepoort Journal of Veterinary Research* 46: 155-63.
- Barnard BJH, Hassel RH. 1981. Rabies in Kudus (*Tragelaphus strepsiceros*) in South West Africa-Namibia. *Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging* 52: 309-14.
- Barnard P, ed. 1998. *Biological diversity in Namibia: a country study*. Windhoek: Namibian Biodiversity Task Force. 332 pp.
- Barrett T. 1999. Morbillivirus infections, with special emphasis on morbilliviruses of carnivores. *Veterinary Microbiology* 69: 3-13.
- Barrett T, Blixenkrone-Moller M, Di Guardo G, Domingo M, Duignan P *et al.*, 1995. Morbilliviruses in aquatic mammals: report on round table discussion. *Veterinary Microbiology* 44: 261-5.
- Barrett T, Visser I, Mamaev L, Goatley L, van Bresse M, Osterhaus A. 1993. Dolphin and Porpoise morbilliviruses are genetically distinct from Phocine distemper virus. *Virology* 193: 1010-2.
- Barrett T, Wohlsein P, Bidewell C, Rowell S. 2004. Canine distemper virus in a Californian sea lion (*Zalophus californianus*). *Veterinary Record* 154: 334-6.
- Bartlett M. 1960. The critical community size for measles in the United States. *Journal of the Royal Statistical Society* 123: 37-44.
- Battilani M, Scagliarini A, Tisato E, Turilli C, Jacoboni I *et al.*, 2001. Analysis of canine parvovirus sequences from wolves and dogs isolated in Italy. *Journal of General Virology* 82: 1555-60.
- Baumgartner W, Alldinger S, Beineke A, Groters S, Herden C *et al.*, 2003. Canine distemper virus--an agent looking for new hosts. *Dtsch Tierarztl Wochenschr* 110: 137-42.
- Beinart W. 1998. The night of the jackal: sheep, pastures and predators in the Cape. *Past and Present* 158: 172-206.
- Bekoff M, Wells M. 1981. Behavioural budgeting by wild coyotes: the influence of food resources and social organisation. *Animal Behaviour* 29: 794-801.
- Bengston J, Boveng P, Franzen U, Have P, Heide-Jorgensen MP, Harkonen T. 1991. Antibodies to canine distemper virus in Antractic seals. *Marine Mammal Science* 7: 85-7.
- Bennett P, Jepson P, Law R, Jones B, Kuiken T *et al.*, 2001. Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. *Environmental Pollution* 112: 33-40.
- Berg W. 1999. *Scent station surveys in Minnesota with reference to predator management*. [www.npwr.usgs.gov/resource/1999/symabs/scent.htm](http://www.npwr.usgs.gov/resource/1999/symabs/scent.htm). Minnesota Department of Natural Resources. Accessed: 2001.
- Berry H. 1993. Surveillance and control of anthrax and rabies in wild herbivores and carnivores in Namibia. *Revue scientifique et technique (International office of epizootics)* 12: 137-46.

- Berry H, ed. 2002. *Cape Cross Past and Present*. Walvis Bay, Namibia
- Berthier K, Langlais M, Auger P, Pontier D. 2000. Dynamics of a feline virus with two transmission modes within exponentially growing host populations. *Proceedings of the Royal Society of London, B* 267: 2049-56.
- Biek R, Zarnke RL, Gillin C, Wild M, Squires JR, Poss M. 2002. Serologic survey for viral and bacterial infections in western populations of Canada lynx (*Lynx canadensis*). *Journal of Wildlife Diseases* 38: 840-5.
- Bingham J, Foggin C. 1993. Jackal rabies in Zimbabwe. *Onderstepoort Journal of Veterinary Research* 60: 365-6.
- Bingham J, Foggin C, Wandeler A, Hill F. 1999. The epidemiology of rabies in Zimbabwe. 2. Rabies in jackals (*Canis adustus* and *Canis mesomelas*). *Onderstepoort Journal of Veterinary Research* 66: 11-23.
- Bingham J, Purchase GK. 2003. Age determination in jackals (*Canis adustus* Sundevall, 1846, and *Canis mesomelas* Schreber, 1778; *Carnivora* : *Canidae*) with reference to the age structure and breeding patterns of jackal populations in Zimbabwe. *African Zoology* 38: 153-60.
- Birkun AJ, Kuiken T, Krivokhizhin S, Haines D, Osterhaus A *et al.*,. 1999. Epizootic of morbilliviral disease in common dolphins (*Delphinus delphis ponticus*) from the Black Sea. *Veterinary Record* 144: 85-92.
- Bishop G. 2001. Increasing dog vaccination coverage in South Africa: is oral vaccination the answer? In *Rabies control in Asia*, ed. B Dodet, F-X Meslin, pp. 105-9. Paris: John Libbey Eurotext.
- Black F. 1966. Measles endemicity in insular populations: critical community size and its evolutionary implication. *Journal of Theoretical Biology* 11: 207-11.
- Blas N, Ortega C, Frankena K, Noordhuizen J, Thrusfield M. 1998. *Win Episcopo*, 2.0. Facultad de Veterinaria. [http://infecepi.unizar.es/pages/ratio/soft\\_uk.htm](http://infecepi.unizar.es/pages/ratio/soft_uk.htm). Zaragoza, Spain.
- Blixenkrone-Moller M, Svansson V, Orvell C, Have P. 1990. Phocid distemper virus - a threat to terrestrial animals? *Veterinary Record* 127: 263-4.
- Bohm J, Blixenkrone-Moller M, Lund E. 1989. A serious outbreak of canine distemper among sled-dogs in northern Greenland. *Arctic Medical Research* 48: 195-203.
- Bolker B, Grenfell B. 1996. Impact of vaccination on the spatial correlation and persistence of measles dynamics. *Proceedings of the National Academy of Science* 93: 12648-53.
- Bornstein S. 1991. Experimental infection of dogs with *Sarcoptes scabiei* derived from naturally infected wild red foxes (*Vulpes vulpes*): clinical observations. *Veterinary Dermatology* 2: 151-9.
- Bornstein S, Morner T, Samuel W. 2001. *Sarcoptes scabiei* and sarcoptic mange. In *Parasitic Diseases of Wild mammals*, ed. W Samuel, M Pybus, A Kocan, pp. 107-19: Manson Publishing, The Veterinary Press.
- Bornstein S, Thebo S, Zakrisson G. 1996. Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of canine sarcoptic mange. *Veterinary Dermatology* 7: 21-8.
- Bornstein S, Zakrisson G. 1994. Humoral antibody response to experimental *Sarcoptes scabiei* var. *vulpes* infection in the dog. *Veterinary Dermatology* 4: 107-10.

- Bourhy H, Sureau P. 1990. *Laboratory methods for rabies diagnosis*. Paris: Institute Pasteur. 197 pp.
- Briggs M, Dubach J, Murnane R. 1998. *Carnivore research in Southern Africa: a project update*. Presented at American Association of Zoo Veterinarians and American Association of Wildlife Veterinarians Joint Conference
- Brooks R. 1990. Survey of the dog population of Zimbabwe and its level of rabies vaccination. *The Veterinary Record* 127: 592-6.
- Brown C. 1996. The outlook for the future. *Namibia Environment* 1: 15-20.
- Buckland S, Anderson D, Burnham K, Laake J. 1993. Distance Sampling: Introductory concepts. In *Distance Sampling: Estimating Abundance of Biological Populations*, pp. 446. London: Chapman and Hall.
- Bulmer M. 1979. *Principles of Statistics*. New York: Dover Publications. 252 pp.
- Butler J. 1998. *The Ecology of Domestic Dogs Canis familiaris in the Communal Lands of Zimbabwe*. University of Zimbabwe, Harare. 236 pp.
- Butler J, Bingham J. 2000. Demography and dog-human relationships of the dog population in Zimbabwean communal lands. *The Veterinary Record* 147: 442-6.
- Butler J, du Toit J. 2002. Diet of free-ranging domestic dogs (*Canis familiaris*) in rural Zimbabwe: implications for wild scavengers on the periphery of wildlife reserves. *Animal Conservation* 5: 29-37.
- Butler JRA, du Toit JT, Bingham J. 2004. Free-ranging domestic dogs (*Canis familiaris*) as predators and prey in rural Zimbabwe: threats of competition and disease to large wild carnivores. *Biological Conservation* 115: 369-78.
- Cabasso V. 1981. Infectious canine hepatitis. In *Infectious Diseases of Wild Mammals*, ed. J Davis, L Karstad, D Trainer. Ames: The Iowa State University Press.
- Cancrini G, Allende E, Favia G, Bornay F, Anton F, Simon F. 2000. Canine dirofilariosis in two cities of southeastern Spain. *Veterinary Parasitology* 92: 81-6.
- Cancrini G, di Regalbono AF, Ricci I, Tessarin C, Gabrielli S, Pietrobelli M. 2003. *Aedes albopictus* is a natural vector of *Dirofilaria immitis* in Italy. *Veterinary Parasitology* 118: 195-202.
- Carmichael L. 1987. Canine parvovirus Type 1 (Minute virus of canines). In *Virus Infections of Carnivores*, ed. M Appel, pp. 63-7. Amsterdam: Elsevier Science Publishers.
- Caro T. 1999. The behaviour-conservation interface. *Tree* 14: 366-7.
- Carpenter MA, Appel MJG, Roelke-Parker ME, Munson L, Hofer H *et al.*, 1998. Genetic characterization of canine distemper virus in Serengeti carnivores. *Veterinary Immunology and Immunopathology* 65: 259-66.
- Catelli E, Cook J, Chesher J, Orbell S, Woods M *et al.*, 1998. The use of virus isolation, histopathology and immunoperoxidase techniques to study the dissemination of a chicken isolate of avian pneumovirus in chickens. *Avian Pathology* 27: 632-40.
- Cattet M, Duignan P, House C, St. Aubin D. 2004. Antibodies to canine distemper and phocine distemper viruses in polar bears from the Canadian Arctic. *Journal of Wildlife Diseases* 40: 338-42.

- Caughely G, Gunn A. 1996. *Conservation Biology in Theory and Practice*. Cambridge, MA: Blackwell Science. 459 pp.
- CDF. 2001. *Canine distemper threatens wildlife of Galápagos*. <http://www.darwinfoundation.org/news/news03150101.html>. Charles Darwin Foundation (CDF). Accessed: 2004.
- Chalmers W, Baxendale W. 1994. A comparison of canine distemper vaccine and measles vaccine for the prevention of canine distemper in young puppies. *Veterinary Record* 135: 349-53.
- Chappuis G. 1995. Control of canine distemper. *Veterinary Microbiology* 44: 351-8.
- Chomel BB, Kasten RW, Chappuis G, Soulier M, Kikuchi Y. 1998. Serological survey of selected canine viral pathogens and zoonoses in grizzly bears (*Ursus arctos horribilis*) and black bears (*Ursus americanus*) from Alaska. *Revue Scientifique et Technique (International Office of Epizootics)* 17: 756-66.
- Choquette L, Kuyt E. 1974. Serological indication of canine distemper and of infectious canine hepatitis in wolves (*Canis lupus* L.) in northern Canada. *Journal of Wildlife Diseases* 10: 321-4.
- Churchill A. 1982. A potency test for inactivated small animal parvovirus vaccines using chicks. *Journal of Biological Standardization* 10: 1-8.
- Cleaveland S. 1996. *The epidemiology of rabies and canine distemper in the Serengeti, Tanzania*. London School of Hygiene and Tropical Medicine, London. 262 pp.
- Cleaveland S. 2003. Emerging infectious diseases of wildlife. *Microbiology Today* 30: 155-6.
- Cleaveland S, Appel MGJ, Chalmers WSK, Chillingworth C, Kaare M, Dye C. 2000. Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. *Veterinary Microbiology* 72: 217-27.
- Cleaveland S, Dye C. 1995. Maintenance of a microparasite infecting several host species: rabies in the Serengeti. *Parasitology* 111 S: S33-47.
- Cleaveland S, Hess G, Dobson A, Laurenson M, McCallum H *et al.*,. 2002. The role of pathogens in biological conservation. In *The Ecology of Wildlife Diseases*, ed. P Hudson, A Rizzoli, B Grenfell, H Heesterbeek, A Dobson, pp. 139-50. Oxford: Oxford University Press.
- Cleaveland S, Laurenson MK, Taylor LH. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philosophical Transactions of the Royal Society, London, Series B: Biological Sciences* 356: 991-9.
- Cleaveland S, Mlengeya T, Kaare M, Haydon D, Laurenson K, Packer C. 2004. The conservation relevance of epidemiological research into carnivore viral diseases in the Serengeti. Manuscript.
- Cochrane K. 1997. *Review of the state of world fishery resources: marine fisheries. Regional reviews: Southeast Atlantic. No.920 FIRM/C920(En)*, Food and Agricultural Organisation (FAO) for the United Nations, Rome.
- Coleman P. 1999. *The epidemiology and control of domestic dog rabies*. University of London, London
- Coleman P, Dye C. 1996. Immunization coverage required to prevent outbreaks of dog rabies. *Vaccine* 14: 185-6.

- Conner MC, Labisky RF, Progulske DRJ. 1983. Scent-station indices as measures of population abundance for bobcats, raccoons, gray foxes, and opossums. *Wildlife Society Bulletin* 11: 146-52.
- Cornwell H, Anderson S, Thompson P, Mayer S, Ross H *et al.*, 1992. The serological response of the common seal (*Phoca vitulina*) and the grey seal (*Halichoerus grypus*) to phocine distemper virus as measured by a canine distemper virus neutralisation test. *The Science of the Total Environment* 115: 99-116.
- Courchamp F, Yoccoz N, Artois M, Pontier D. 1998. At-risk individuals in Feline Immunodeficiency Virus epidemiology: evidence from a multivariate approach in a natural population of domestic cats (*Felis catus*). *Epidemiology and Infection* 121: 227-36.
- Courtenay O, Quinnell RJ, Chalmers WSK. 2001. Contact rates between wild and domestic canids: no evidence of parvovirus or canine distemper virus in crab-eating foxes. *Veterinary Microbiology* 81: 9-19.
- Courtenay O, Quinnell RJ, Garcez LM, Shaw JJ, Dye C. 2002. Infectiousness in a cohort of Brazilian dogs: Why culling fails to control visceral leishmaniasis in areas of high transmission. *Journal of Infectious Diseases* 186: 1314-20.
- Courtin F, Carpenter TE, Paskin RD, Chomel BB. 2000. Temporal patterns of domestic and wildlife rabies in central Namibia stock-ranching area, 1986-1996. *Preventive Veterinary Medicine* 43: 13-28.
- Cowel R. 1999. *Diagnostic cytology and haematology for the dog and cat*. London: Mosby. 320 pp.
- Crawley M. 1993. GLIM for Ecologists. pp. 188-210. Oxford: Blackwell Scientific Publications.
- Creel S, Creel N. 1998. Six ecological factors that may limit African wild dogs, *Lycaon pictus*. *Animal Conservation* 1: 1-9.
- Creel S, Creel NM, Matovelo J, Mtambo M, Batamuzi E, Cooper EJ. 1995. The effects of anthrax on endangered African wild dogs (*Lycaon pictus*). *Journal of Zoology, London* 236: 199-209.
- Creel S, Creel NM, Munson L, Sanderlin D, Appel MJ. 1997. Serosurvey for selected viral diseases and demography of African wild dogs in Tanzania. *Journal of Wildlife Diseases* 33: 823-32.
- Curtis C. 2001. Evaluation of a commercially available enzyme-linked immunosorbent assay for the diagnosis of canine sarcoptic mange. *Veterinary Record* 148: 238-9.
- Cypher B. 1997. Effects of radio-collars on San-Joaquin kit foxes. *Journal of Wildlife Management* 61: 1412-23.
- Cypher BL, Frost N. 1999. Condition of San Joaquin kit foxes in urban and exurban habitats. *Journal of Wildlife Management* 63: 930-8.
- Cypher BL, Scrivner JH, Hammer KL, O'Farrell TP. 1998. Viral antibodies in coyotes from California. *Journal of Wildlife Diseases* 34: 259-64.
- Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife--threats to biodiversity and human health. *Science* 287: 443-9.
- DCW. 1997. *Digital Charts of the World (DCW): Namibia (Network, PONET)*. <http://www.maproom.psu.edu/dcw/>. Environmental Systems Research Institute. Accessed: 2004.



- De Koeijer A, Diekmann O, Reijnders P. 1998. Modelling the spread of phocine distemper virus among harbour seals. *Bulletin of Mathematical Biology* 60: 585-96.
- De Lisle GW, Bengis RG, Schmitt SM, O'Brien DJ. 2002. Tuberculosis in free-ranging wildlife: detection, diagnosis and management. *Revue Scientifique et Technique (International office of epizootics)* 21: 317-34.
- De Swart R, Harder T, Ross P, Vos H, Osterhaus A. 1995a. Morbilliviruses and morbillivirus diseases of marine mammals. *Infectious Agents and Disease* 4: 125-30.
- De Swart RL, Ross PS, Timmerman HH, Vos HW, Reijnders PJH *et al.*,. 1995b. Impaired cellular immune-response in harbor seals (*Phoca vitulina*) feeding on environmentally contaminated herring. *Clinical and Experimental Immunology* 101: 480-6.
- De Swart RL, Ross PS, Vos JG, Osterhaus A. 1996. Impaired immunity in harbour seals (*Phoca vitulina*) exposed to bioaccumulated environmental contaminants: Review of a long-term feeding study. *Environmental Health Perspectives* 104: 823-8.
- De Villiers D, Roux J. 1992. Mortality of newborn pups of the South African fur seal *Arctocephalus pusillus pusillus* in Namibia. *South African Journal of Marine Science* 12: 881-9.
- Dean A, Dean J, Coulombier D, Brendel K, Smith D *et al.*,. 1995. *Epi Info: A word-processing, database, and statistics program for public health on IBM-compatible microcomputers*, 6.0. Centres for Disease Control and Prevention. Atlanta.
- Decaro N, Pratelli A, Campolo M, Elia G, Martella V *et al.*,. 2004. Quantitation of canine coronavirus RNA in the faeces of dogs by TaqMan RT-PCR. *Journal of Virological Methods* 119: 145-50.
- Deem SL, Noss AJ, Cuellar RL, Villarroel R, Linn MJ, Forrester DJ. 2002. Sarcoptic mange in free-ranging pampas foxes in the Gran Chaco, Bolivia. *Journal of Wildlife Diseases* 38: 625-8.
- Deem SL, Spelman LH, Yates RA, Montali RJ. 2000. Canine distemper in terrestrial carnivores: a review. *Journal of Zoo and Wildlife Medicine* 31: 441-51.
- Dentlinger L. 2004. *New project to bolster National Parks system*. <http://www.namibian.com.na/2004/June/national/0448A0CDE9.html>. Accessed: 2004.
- Diefenbach D, Conroy M, Warren R, James W, Baker L. 1994. A test of the scent-station survey technique for Bobcats. *Journal of Wildlife Management* 58: 10-7.
- Dierauf L, Lowenstine LJ, Jerome C. 1981. Viral hepatitis (adenovirus) in a Californian sea lion. *Journal of the American Veterinary Medical Association* 179: 1194-7.
- Dietz R, Ansen C, Have P. 1989a. Clue to seal epizootic? *Nature* 338: 627.
- Dietz R, Heide-Jorgenson M, Harkonen T. 1989b. Mass death of harbour seals *Phoca vitulina* in Europe. *Ambio* 18: 258-64.
- Dillaha JA, Yang ZH, Ijaz K, Eisenach KD, Cave MD *et al.*,. 2002. Transmission of *Mycobacterium tuberculosis* in a rural community, Arkansas, 1945-2000. *Emerging Infectious Diseases* 8: 1246-8.
- Dixon K, Chapman J. 1980. Harmonic mean measure of animal activity areas. *Ecology* 61: 1040-4.



- Dobson A. 1995. Ecology and epidemiology of rinderpest virus. In *Serengeti II: Dynamics, Management and Conservation of an Ecosystem*, ed. A Sinclair, P Arcese, pp. 485-505. Chicago: The University of Chicago Press.
- Dobson A, Hudson P. 1986. Parasites, diseases and the structure of ecological communities. *Tree* 1: 11-4.
- Domingo M, Ferrer L, Pumarola M, Marco A, Plana J *et al.*,. 1990. Morbillivirus in dolphins. *Nature* 348: 21.
- Domingo M, Vilafranca M, Visa J, Prats N, Trudgett A, Visser I. 1995. Evidence for chronic morbillivirus infection in the Mediterranean striped dolphin (*Stenella coeruleoalba*). *Veterinary Microbiology* 44: 229-39.
- Donnelly CA, Woodroffe R, Cox DR, Bourne J, Gettinby G *et al.*,. 2003. Impact of localized badger culling on tuberculosis incidence in British cattle. *Nature* 426: 834-7.
- Drobniewski FA, Gibson A, Ruddy M, Yates MD. 2003. Evaluation and utilization as a public health tool of a national molecular epidemiological tuberculosis outbreak database within the United Kingdom from 1997 to 2001. *Journal of Clinical Microbiology* 41: 1861-8.
- Duignan PJ, House C, Geraci JR, Duffy N, Rima BK *et al.*,. 1995. Morbillivirus infection in cetaceans of the western Atlantic. *Veterinary Microbiology* 44: 241-9.
- Dunbar MR, Cunningham MW, Roof JC. 1998. Seroprevalence of selected disease agents from free-ranging black bears in Florida. *Journal of Wildlife Diseases* 34: 612-9.
- Dye C, Barlow N, Begon M, Bowers R, Bolker B *et al.*,. 1995. Microparasite report group: persistence of microparasites in natural populations. In *Ecology of Infectious Diseases in Natural Populations*, ed. B Grenfell, A Dobson, pp. 123-43. Cambridge: Cambridge University Press.
- Eckersley G, Hohn E, Reyers F, Turner G, Wolmarans L. 1992. A comparison between the disease status of hospitalised dogs from developed and those from developing communities. *Journal of the South African Veterinary Association* 63: 2-6.
- Edmunds W, O'Callaghan C, Nokes D. 1997. Who mixes with whom? A method to determine the contact patterns of adults that may lead to the spread of airborne infections. *Proceedings of the National Academy of Science, B* 264: 949-57.
- Ek-Kommonen C, Rudback E, Anttila M, Aho M, Huovilainen A. 2003. Canine distemper of vaccine origin in European mink, *Mustela lutreola* - a case report. *Veterinary Microbiology* 92: 289-93.
- Ek-Kommonen C, Sihvonen L, Pekkanen K, Rikula U, Nuotio L. 1997. Outbreak of canine distemper in vaccinated dogs in Finland. *Veterinary Record* 141: 380-3.
- Estes S, Estes J. 1993. Scabies research: another dimension. *Seminars in Dermatology* 12: 34-8.
- Fairchild G, Wyman M, Donovan E. 1967. Fluorescent antibody technique as a diagnostic test for canine distemper infection: detection of viral antigen in epithelial tissues of experimentally infected dogs. *American Journal of Veterinary Research* 28: 761-8.
- Ferguson J, Galpin S, De Wet M. 1988. Factors affecting the activity patterns of black-backed jackals *Canis mesomelas*. *Journal of Zoology, London* 214: 55-69.

- Ferguson J, Nel J, De Wet M. 1983. Social organisation and movement patterns of black-backed jackals *Canis mesomelas* in South Africa. *Journal of Zoology, London* 199: 487-502.
- Fiorello CV, Deem SL, Gompper ME, Dubovi EJ. 2004. Seroprevalence of pathogens in domestic carnivores on the border of Madidi National Park, Bolivia. *Animal Conservation* 7: 45-54.
- Follmann EH, Garner G, Evermann JF, McKeirnan AJ. 1996. Serological evidence of morbillivirus infection in polar bears (*Ursus maritimus*) from Alaska and Russia. *The Veterinary Record* 138: 615-8.
- Ford R, Vaden S. 1998. Canine infectious tracheobronchitis. In *Infectious Diseases of the Dog and Cat*, ed. G Greene, pp. 33-8. Philadelphia: WB Saunders Company.
- Forsyth MA, Kennedy S, Wilson S, Eybatov T, Barrett T. 1998. Canine distemper virus in a Caspian seal. *Veterinary Record* 143: 662-4.
- Frair J, Nielsen S, Merrill E, Lele S, Boyce M *et al.*, 2004. Removing GPS collar bias in habitat selection studies. *Journal of Applied Ecology* 41: 201-12.
- Frisk AL, Konig M, Moritz A, Baumgartner W. 1999. Detection of canine distemper virus nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. *Journal of Clinical Microbiology* 37: 3634-43.
- Frolich K, Czupalla O, Haas L, Hentschke J, Dedek J, Fickel J. 2000. Epizootiological investigations of canine distemper virus in free-ranging carnivores from Germany. *Veterinary Microbiology* 74: 283-92.
- Fromont E, Courchamp F, Artois M, Pontier D. 1997. Infection strategies of retroviruses and social grouping of domestic cats. *Canadian Journal of Zoology* 75: 1994-2002.
- Fuller T, Biknevicius A, Kat P, Van Valkenburgh B, Wayne R. 1989. The ecology of three sympatric jackal species in the Rift Valley of Kenya. *African Journal of Ecology* 27: 313-23.
- Funk S, Fiorello C, Cleaveland S, Gomper M. 2001. The role of disease in carnivore ecology and conservation. In *Carnivore conservation*, ed. J Gittleman, S Funk, D Macdonald, R Wayne, pp. 443-66. Cambridge: Cambridge University Press.
- Gagneux P, Woodruff D, Boesch C. 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. *Molecular Ecology* 6: 861-8.
- Galbusera P, Githiru M, Lens L, Matthysen E. 2004. Genetic equilibrium despite habitat fragmentation in an Afrotropical bird. *Molecular Ecology* 13: 1409-21.
- Gascoyne S, King A, Laurenson M, Borner M, Schildger B, Barrat J. 1993. Aspects of rabies infection and control in the conservation of the African wild dog (*Lycaon pictus*) in the Serengeti region, Tanzania. *Onderstepoort Journal of Veterinary Research* 60: 415-20.
- Gaskell R, Willoughby K. 1999. Herpesviruses of carnivores. *Veterinary Microbiology* 69: 73-88.
- Gerber J, Roletto J, Morgan L, Smith D, Gage L. 1993. Findings in pinnipeds stranded along the central and northern California coast, 1984-1990. *Journal of Wildlife Diseases* 29: 423-33.
- Gerber L, Hilborn R. 2001. Catastrophic events and recovery from low densities in populations of otariids: implications for risk of extinction. *Mammal Review* 31: 131-50.

- Gese E, Schultz R, Rongstad O, Andersen D. 1991. Prevalence of antibodies against canine parvovirus and canine distemper virus in wild coyotes in southeastern Colorado. *Journal of Wildlife Diseases* 27: 320-3.
- Ginsberg J. 2001. Setting priorities for carnivore conservation: what makes carnivores different? In *Carnivore Conservation*, ed. J Gittleman, S Funk, D Macdonald, R Wayne, pp. 498-523. Cambridge: Cambridge University Press.
- Ginsberg J, Macdonald D, eds. 1990. *Foxes, wolves, jackals and dogs*. Gland, Switzerland: IUCN Publications. 116 pp.
- Goldstein T, Gulland F, Aldridge B, Harvey J, Rowles T *et al.*,. 2003. Antibodies to phocine herpesvirus-1 are common in north American Harbour seals (*Phoca vitulina*). *Journal of Wildlife Disease* 39: 487-94.
- Goltsman M, Kruchenkova E, Macdonald D. 1996. The Mednyi Arctic foxes: treating a population imperilled by disease. *Oryx* 30: 251-8.
- Goossens B, Waits L, Taberlet P. 1998. Plucked hair samples as a source of DNA: reliability of dinucleotide microsatellite genotyping. *Molecular Ecology* 7: 1237-41.
- Gordon J, Angrick E. 1986. Canine parvovirus: environmental effects on infectivity. *American Journal of Veterinary Research* 47: 1464-7.
- Gorham J. 1966. The epizootiology of distemper. *Journal of the American Veterinary Medical Association* 149: 610-22.
- Goth R. 1999. *Rhipicephalus sanguineus* (Ixodidae): frequency of infestation and ehrlichial infections transmitted by this tick species in dogs in Germany; an epidemiological study and consideration. *Wiener Tierärztliche Monatsschrift* 86: 49-56.
- Grachev M, Kumarev V, Mamaev L, Zorin V, Baranova L *et al.*,. 1989. Distemper virus in Lake Baikal seals. *Nature* 338: 209.
- Greene G. 1998. Infectious canine hepatitis and canine acidophil cell hepatitis. In *Infectious Diseases of the Dog and Cat*, ed. G Greene, pp. 22-9. Philadelphia: WB Saunders Company.
- Greene G, Appel M. 1998. Canine distemper. In *Infectious Diseases of the Dog and Cat*, ed. G Greene, pp. 9-22. Philadelphia: WB Saunders Company.
- Grenfell B, Anderson R. 1985. The estimation of age-related rates of infection from case notifications and serological data. *Journal of Hygiene Cambridge* 95: 419-36.
- Grenfell B, Dobson A, eds. 1995. *Ecology of Infectious Diseases in Natural Populations*. Cambridge: Cambridge University Press. 521 pp.
- Griffin C, Kwochka K, MacDonald J. 1993. *Current Veterinary Dermatology: The Science and Art of Therapy*. London: Mosby. 378 pp.
- Haas L, Hofer H, East M, Wohlsein P, Liess B, Barrett T. 1996. Canine distemper virus infection in Serengeti spotted hyenas. *Veterinary Microbiology* 49: 147-52.
- Hahn BH, Shaw GM, De Cock KM, Sharp PM. 2000. AIDS as a zoonosis: scientific and public health implications. *Science* 287: 607-14.
- Hall A, Pomeroy P, Harwood J. 1992. The descriptive epizootiology of phocine distemper virus in the UK during 1988/89. *Science of the Total Environment* 115: 31-44.

- Hamir AN, Raju N, Hable C, Rupprecht CE. 1992. Retrospective study of testicular degeneration in raccoons with canine distemper infection. *Journal of Veterinary Diagnostic Investigation* 4: 159-63.
- Harder T, Kenter M, Appel M, Roelke-Parker M, Barrett T, Osterhaus A. 1995. Phylogenetic evidence of canine distemper virus in Serengeti's lions. *Vaccine* 13: 521-3.
- Harder T, Willhaus R, Frey H-R, Leiss B. 1990. Morbillivirus infections of seals during the 1988 epidemic in the Bay of Heligoland. III. Transmission studies of cell culture-propagated phocine distemper virus in harbour seals (*Phoca vitulina*) and a grey seal (*Halichoerus grypus*): clinical, virological and serological results. *Journal of Veterinary Medicine, Series B* 39: 641-50.
- Harder TC, Kenter M, Vos H, Sieblink K, Huisman W *et al.*,. 1996. Canine distemper virus from diseased large felids: biological properties and phylogenetic relationships. *Journal of General Virology* 77: 397-405.
- Harder TC, Osterhaus A. 1997. Canine distemper virus - A morbillivirus in search of new hosts? *Trends in Microbiology* 5: 120-4.
- Harding K, Harkonen T, Caswell C. 2002. The 2002 European seal plague: epidemiology and population consequences. *Ecology Letters* 5: 727-32.
- Harkonen T, Harding K. 1999. Age and sex specific behaviour in harbour seals leads to biased estimates of vital population parameters. *Journal of Applied Ecology* 36
- Harrison T, Mazet J, Holekamp K, Dubovi EJ, Engh A *et al.*,. 2004. Antibodies to canine and feline viruses in spotted hyenas (*Crocuta crocuta*) in the Masai Mara National Reserve. *Journal of Wildlife Diseases* 40: 1-10.
- Harvell C, Kim K, Burkholder J, Colwell R, Epstein P *et al.*,. 1999. Emerging marine diseases-climate links and anthropogenic factors. *Science* 285: 1505-10.
- Hawkins C, Laurenson M, Haydon D. 2002. Does behavioural ecology have a role in the management of infectious diseases in wildlife? Manuscript.
- Haydon D, Cleaveland S, Taylor L, Laurenson M. 2002. Identifying reservoirs of infection: a conceptual and practical challenge. *Emerging Infectious Diseases* 8: 1468-73.
- Heaney J, Barrett T, Cosby SL. 2002. Inhibition of *in vitro* leukocyte proliferation by morbilliviruses. *Journal of Virology* 76: 3579-84.
- Heesterbeek J, Roberts M. 1995. Mathematical models for microparasites of wildlife. In *Ecology of Infectious Diseases in Natural Populations*, ed. B Grenfell, A Dobson, pp. 90-122. Cambridge: Cambridge University Press.
- Heide-Jorgenson M, Harkonen T. 1992. Epizootiology of the seal disease in the eastern North Sea. *Journal of Applied Ecology* 29: 99-107.
- Henderson G, Trudgett A, Lyons C, Ronald K. 1992. Demonstration of antibodies in archival sera from Canadian seals reactive with a European isolate of phocine distemper virus. *The Science of the Total Environment* 115: 93-8.
- Henschel J. 2001. Jackals and their importance for the dispersal of the seeds of the economically important !Nara melon of the Namibian desert. Pers. comm.

- Hernandez M, Robinson I, Aguilar A, Gonzalez L, Lopez-Jurado L *et al.*, 1998. Did algal toxins cause monk seal mortality? *Nature* 393: 28-9.
- Hidalgo-Mihart MG, Cantu-Salazar L, Lopez-Gonzalez CA, Fernandez EC, Gonzalez-Romero A. 2004. Effect of a landfill on the home range and group size of coyotes (*Canis latrans*) in a tropical deciduous forest. *Journal of Zoology* 263: 55-63.
- Hiscocks K, Perrin M. 1987. Feeding observations and diet of black-backed jackals in an arid coastal environment. *South African Journal of Wildlife Research* 17: 55-8.
- Hiscocks K, Perrin M. 1988. Home range and movements of black-backed jackals at Cape Cross Seal Reserve, Namibia. *South African Journal of Wildlife Research* 18: 97-9.
- Hoff G, Bigler W. 1974. Epizootic of canine distemper virus infection among urban raccoons and gray foxes. *Journal of Wildlife Diseases* 10: 423-8.
- Hofmeyr M. 2001. *Introduction of the African wild dog (Lycaon pictus) to game reserves.* <http://bigfive.jl.co.za/wilddogs.htm>. Big Five Veterinary Pharmaceutical Company (Pty) Ltd. Accessed: 2004.
- Hofmeyr M, Hofmeyr D, Nel L, Bingham J. 2004. A second outbreak of rabies in African wild dogs (*Lycaon pictus*) in Madikwe Game Reserve, South Africa, demonstrating the efficacy of vaccination against natural rabies challenge. *Animal Conservation* 7: 193-8.
- Holzman S, Conroy M, Davidson W. 1992. Diseases, parasites and survival of coyotes in South-Central Georgia. *Journal of Wildlife Diseases* 28: 572-80.
- Hoskins J. 1998. Canine viral enteritis. In *Infectious Diseases of the Dog and Cat*, ed. G Greene, pp. 40-9. Philadelphia: WB Saunders Company.
- Hubschle OJ. 1988. Rabies in the kudu antelope (*Tragelaphus strepsiceros*). *Reviews of Infectious Diseases* 10 Suppl 4: S629-33.
- Hulbert I, French J. 2001. The accuracy of GPS for wildlife telemetry and habitat mapping. *Journal of Applied Ecology* 38: 869.
- Ikeda Y, Nakamura K, Miyazawa T, Tohya Y, Takahashi E, Mochizuki M. 2002. Feline host range of canine parvovirus: recent emergence of new antigenic types in cats. *Emerging Infectious Diseases* 8: 341-6.
- Jauniaux T, Charlier G, Desmecht M, Haelters J, Jacques T *et al.*, 2000. Pathological findings in two fin whales (*Balaenoptera physalus*) with evidence of morbillivirus infection. *Journal of Comparative Pathology* 123: 198-201.
- Jensen T, van de Bildt M, Dietz HH, Andersen TH, Hammer AS *et al.*, 2002. Another phocine distemper outbreak in Europe. *Science* 297: 209.
- Jepson P, Bennett P, Allchin C, Robin J, Kuiken T *et al.*, 1999. Investigating potential associations between chronic exposure to polychlorinated biphenyls and infectious disease mortality in harbour porpoises from England and Wales. *The Science of the Total Environment* 243/244: 339-48.
- Jetz W, Carbone C, Fulford J, Brown JH. 2004. The scaling of animal space use. *Science* 306: 266-8.
- Johnson M, Boyd D, Pletscher D. 1994. Serologic investigations of canine parvovirus and canine distemper in relation to wolf (*Canis lupus*) pup mortalities. *Journal of Wildlife Diseases* 30: 270-3.

- Johnson S, Nolan S, Gulland F. 1998. Antimicrobial susceptibility of bacteria isolated from pinnipeds stranded in central and northern California. *Journal of Zoo and Wildlife Medicine* 29: 288-94.
- Kasuya E. 2004. Angular transformation - another effect of different sample sizes. *Ecological Research* 19: 165-7.
- Kaunda S. 2001. Capture and chemical immobilization of black-backed jackals at Mokolodi Nature Reserve, Botswana. *South African Journal of Wildlife Research* 31: 43-7.
- Kaunda S, Skinner J. 2003. Black-backed jackal diet at Mokolodi Nature Reserve, Botswana. *African Journal of Ecology* 41: 39-46.
- Kaunda SKK. 2000. Activity patterns of black-backed jackals at Mokolodi Nature Reserve, Botswana. *South African Journal of Wildlife Research* 30: 157-62.
- Kennedy S, Kuiken T, Jepson PD, Deaville R, Forsyth M *et al.*,. 2000. Mass die-off of Caspian seals caused by canine distemper virus. *Emerging Infectious Diseases* 6: 637-9.
- Kennedy S, Smyth J, Cush P, Duignan P, Platterton M *et al.*,. 1988. Viral distemper now found in porpoises. *Nature* 336: 21.
- Kim MK, Kim CH, Yeom BW, Park SH, Choi SY, Choi JS. 2002. The first human case of hepatic dirofilaria. *Journal of Korean Medical Science* 17: 686-90.
- Kim Y, Cho K, Youn H, Yoo H, Han H. 2001. Detection of canine distemper virus (CDV) through one step RT-PCR combined with nested PCR. *Journal of Veterinary Science* 2: 59-63.
- Kingdon J. 1997. *The Kingdon Guide to African Mammals*. London: Academic Press. 464 pp.
- Kissui BM, Packer C. 2004. Top-down population regulation of a top predator: lions in the Ngorongoro Crater. *Proceedings of the Royal Society of London Series B-Biological Sciences* 271: 1867-74.
- Kitala P, McDermott J, Kyule M, Gathuma J, Perry B, Wandeler A. 2001. Dog ecology and demography information to support the planning of rabies control in Machakos District, Kenya. *Acta Tropica* 78: 217-30.
- Kock R, Chalmers W, Mwanzia J, Chillingworth C, Wambua J *et al.*,. 1998. Canine distemper antibodies in lions of the Masai Mara. *Veterinary Record* 142: 662-5.
- Kohn M, York E, Karmradt D, Haught G, Sauvajot R, Wayne R. 1999. Estimating population size by genotyping faeces. *Proceedings of the Royal Society of London Series B-Biological Sciences* 266: 657-63.
- Kok OB. 1996. Diet composition of different carnivores in the Free State, South Africa. *South African Journal of Science* 92: 393-8.
- Krakowka S, Koestner A. 1976. Age-related susceptibility to infection with canine distemper virus in gnotobiotic dogs. *Journal of Infectious Diseases* 134: 629-32.
- Kumar P, Garg SK, Gupta PK, Bandyopadhyay SK, Singh R, Shrivastava S. 2003. Detection of canine parvovirus DNA by polymerase chain reaction. *Indian Journal of Animal Sciences* 73: 573-5.
- Kvitek R, Bretz C. 2004. Harmful algal bloom toxins protect bivalve populations from sea otter predation. *Marine Ecology Progress Series* 271: 233-43.



- Laddomada A. 2000. Incidence and control of CSF in wild boar in Europe. *Veterinary Microbiology* 73: 121-30.
- Lamprecht J. 1978. On diet, foraging behaviour and interspecific food competition of jackals in the Serengeti National Park, East Africa. *Zeitschrift Fur Saugetierkunde-International Journal of Mammalian Biology* 43: 210-23.
- Laurenson K, Esterhuysen J, Stander P, Van Heerden J. 1997a. Aspects of rabies epidemiology in Tsumkwe District, Namibia. *Onderstepoort Journal of Veterinary Research* 64: 39-45.
- Laurenson K, Van Heerden J, Stander P, Van Vuuren MJ. 1997b. Seroepidemiological survey of sympatric domestic and wild dogs (*Lycaon pictus*) in Tsumkwe District, north-eastern Namibia. *Onderstepoort Journal of Veterinary Research* 64: 313-6.
- Laurenson M, Cleaveland S, Artois M, Woodroffe R. 2004. Assessing and managing infectious disease threats to canids. In *Canids: Foxes, Wolves, Jackals and Dogs. Status Survey and Conservation Action Plan.*, ed. C Sillero-Zubiri, M Hoffmann, D Macdonald, pp. 246-55. Gland: IUCN/SSC Canid Specialist Group.
- Laurenson MK, Sillero-Zubiri C, Thompson H, Shiferaw F, Thirgood S, Malcolm J. 1998. Disease as a threat to endangered species: Ethiopian wolves, domestic dogs, and canine pathogens. *Animal Conservation* 1: 273-80.
- Leisewitz AL, Carter A, van Vuuren M, van Blerk L. 2001. Canine distemper infections, with special reference to South Africa, with a review of the literature. *Journal of the South African Veterinary Association* 72: 127-36.
- Liebana E, Garcia-Migura L, Clouting C, Clifton-Hadley FA, Breslin M, Davies RH. 2003. Molecular fingerprinting evidence of the contribution of wildlife vectors in the maintenance of *Salmonella enteritidis* infection in layer farms. *Journal of Applied Microbiology* 94: 1024-9.
- Lindeque PM, Turnbull PCB. 1994. Ecology and epidemiology of anthrax in the Etosha National Park, Namibia. *Onderstepoort Journal of Veterinary Research* 61: 71-83.
- Lindstorm E, Andren H, Angelstam P, Cederlund G, Hornfeldt B *et al.*. 1994. Disease reveals the predator: sarcoptic mange, red fox predation, and prey populations. *Ecology* 75: 1042-9.
- Lindstorm E, Morner T. 1985. The spreading of sarcoptic mange among Swedish red foxes (*Vulpes vulpes*) in relation to fox population dynamics. *Revue d'Ecologie (la Terre et la Vie)* 40: 211-6.
- Linhart S, Knowlton F. 1975. Determining the relative abundance of coyotes by scent station lines. *Wildlife Society Bulletin* 3: 119-24.
- Little S, Davidson W, Rakich P, Nixon T, Bounous D, Nettles V. 1998a. Responses of red foxes to first and second infection with *Sarcoptes scabiei*. *Journal of Wildlife Management* 34: 600-11.
- Little SE, Davidson WR, Howerth EW, Rakich PM, Nettles VF. 1998b. Diseases diagnosed in red foxes from the southeastern United States. *Journal of Wildlife Diseases* 34: 620-4.
- Lombaard L. 1971. Age determination and growth curves in the black-backed jackal, *Canis mesomelas* Schreber, 1775 (*Carnivora: canidae*). *Annals of the Transvaal Museum* 27: 135-75.



- Loveridge A, Macdonald D. 2001. Seasonality in spatial organization and dispersal of sympatric jackals (*Canis mesomelas* and *C. latrans*): implications for rabies management. *Journal of Zoology, London* 253: 101-11.
- Loveridge AJ, Macdonald DW. 2002. Habitat ecology of two sympatric species of jackals in Zimbabwe. *Journal of Mammalogy* 83: 599-607.
- Loveridge AJ, Macdonald DW. 2003. Niche separation in sympatric jackals (*Canis mesomelas* and *Canis adustus*). *Journal of Zoology* 259: 143-53.
- Loveridge AJ, Nel JAJ. 2004. Black-backed jackal (*Canis mesomelas*). In *Canids: Foxes, Wolves, Jackals and Dogs. Status Survey and Conservation Action Plan.*, ed. C Sillero-Zubiri, M Hoffmann, D Macdonald, pp. 161-6. Gland: IUCN/SSC Canid Specialist Group.
- Lower K, Medleau L, Hnilica K, Bigler B. 2001. Evaluation of an enzyme-linked immunosorbant assay (ELISA) for the serological diagnosis of sarcoptic mange in dogs. *Veterinary Dermatology* 12: 315-20.
- Lucas Z, Stobo W. 2000. Shark-inflicted mortality on a population of harbour seals (*Phoca vitulina*) at Sable Island, Nova Scotia. *Journal of Zoology* 252: 405-14.
- Lyles A, Dobson A. 1993. Infectious disease and intensive management: population dynamics, threatened hosts, and their parasites. *Journal of Zoo and Wildlife Medicine* 24: 315-26.
- Macchioni G. 1995. *Ancylostoma genettae*, *A. protelesis*, *A. somaliense*: three new species from wild *Carnivora* in the Somali Republic. *Parassitologia* 37: 219-28.
- Macdonald D. 1979. The flexible social system of the golden jackal, *Canis aureus*. *Behavioural Ecology and Sociobiology* 5: 17-38.
- Macdonald D. 1980. *Rabies and wildlife: a biologist's perspective*. Oxford: Oxford University Press. 151 pp.
- Macdonald D. 1993. Rabies and wildlife: a conservation problem? *Onderstepoort Journal of Veterinary Research* 60: 351-5.
- Macdonald D. 1996. Dangerous liaisons and disease. *Nature* 379: 400-1.
- Macdonald D, ed. 2001. *The New Encyclopaedia of Mammals*. Oxford: Oxford University Press. 930 pp.
- Macdonald D, Bacon P. 1982. Fox society, contact rate and rabies epizootiology. *Comparative Immunology and Microbiology of Infectious Diseases* 5: 247-56.
- Macdonald DW, Courtenay O, Forbes S, Mathews F. 1999. The red fox (*Vulpes vulpes*) in Saudi Arabia: loose-knit groupings in the absence of territoriality. *Journal of Zoology* 249: 383-91.
- Machida N. 1993. Pathology and epidemiology of canine distemper in raccoon dogs (*Nyctereutes procyonoides*). *Journal of Comparative Pathology* 108: 383-92.
- MacPherson C, Meslin F-X, Wandeler A, eds. 2000. *Dogs, zoonoses, and public health*. New York: CABI Publishing. 382 pp.
- MacPherson CN, Karstad L, Stevenson P, Arundel JH. 1983. Hydatid disease in the Turkana District of Kenya. III. The significance of wild animals in the transmission of *Echinococcus granulosus*, with particular reference to Turkana and Masailand in Kenya. *Annals of Tropical Medicine and Parasitology* 77: 61-73.

- Maehr DS, Greiner EC, Lanier JE, Murphy D. 1995. Notoedric mange in the Florida panther (*Felis concolor coryi*). *Journal of Wildlife Diseases* 31: 251-4.
- Maes RK, Wise AG, Fitzgerald SD, Ramudo A, Kline J *et al.*,. 2003. A canine distemper outbreak in Alaska: diagnosis and strain characterization using sequence analysis. *Journal of Diagnostic Veterinary Investigation* 15: 213-20.
- Mahy B, Barrett T, Evans S, Anderson E, Bostock C. 1988. Characterisation of a seal morbillivirus. *Nature* 336: 115.
- Maia OB, Gouveia AMG. 2001. Serologic response of maned wolves (*Chrysocyon brachyurus*) to canine distemper virus and canine parvovirus vaccination. *Journal of Zoo and Wildlife Medicine* 32: 78-80.
- Mamaev L, Visser I, Belikov S, Denikina N, Harder TC *et al.*,. 1996. Canine distemper virus in Lake Baikal seals (*Phoca sibirica*). *Veterinary Record* 138: 437-9.
- Mamaev LV, Denikina NN, Belikov SI, Volchkov VE, Visser IKG *et al.*,. 1995. Characterisation of morbilliviruses isolated from Lake Baikal seals (*Phoca sibirica*). *Veterinary Microbiology* 44: 251-9.
- Martella V, Pratelli A, Cirone F, Zizzo N, Decaro N *et al.*,. 2002. Detection and genetic characterization of canine distemper virus (CDV) from free-ranging red foxes in Italy. *Molecular and Cellular Probes* 16: 77-83.
- McCallum H, Harvell D, Dobson A. 2003. Rates of spread of marine pathogens. *Ecology Letters* 6: 1062-7.
- McElroy PD, Sterling TR, Driver CR, Kreiswirth B, Woodley CL *et al.*,. 2002. Use of DNA fingerprinting to investigate a multiyear, multistate tuberculosis outbreak. *Emerging Infectious Diseases* 8: 1252-6.
- McIntyre C. 1998. *Namibia: The Bradt Travel Guide*. Chalfont St. Peter: Bradt Publications. 478 pp.
- McKenzie A. 1993. Biology of the black-backed jackal *Canis mesomelas* with reference to rabies. *Onderstepoort Journal of Veterinary Research* 60: 367-71.
- Mech D, Goyal A. 1995. Effects of canine parvovirus on gray wolves in Minnesota. *Journal of Wildlife Management* 59: 565-70.
- Miller DS, Covell DF, McLean RG, Adrian WJ, Niezgoda M *et al.*,. 2000. Serologic survey for selected infectious disease agents in swift and kit foxes from the western United States. *Journal of Wildlife Diseases* 36: 798-805.
- Minnaar WN, Krecek RC. 2001. Helminths in dogs belonging to people in a resource-limited urban community in Gauteng, South Africa. *Onderstepoort Journal of Veterinary Research* 68: 111-7.
- Minnaar WN, Krecek RC, Fourie LJ. 2002. Helminths in dogs from a peri-urban resource-limited community in Free State Province, South Africa. *Veterinary Parasitology* 107: 343-9.
- Minnaar WN, Krecek RC, Rajput JI. 1999. Helminth parasites of dogs from two resource-limited communities in South Africa. *Journal of the South African Veterinary Association* 70: 92-4.
- Moehlman P. 1983. Socioecology of silverbacked and golden jackals (*Canis mesomelas* and *Canis aureus*). In *Recent Advances in the Study of Mammalian Behaviour*, ed. J Eisenberg, D Kleiman, pp. 423-38

- Moehlman PD. 1979. Jackal helpers and pup survival. *Nature* 277: 382-3.
- Montali R, Bartz C, Bush M. 1987. Canine distemper virus. In *Virus Infections of Carnivores*, ed. M Appel, pp. 437-43. Amsterdam: Elsevier Science Publishers.
- Montali R, Bartz C, Bush M. 1991. Canine distemper virus. In *Virus infections of carnivores*, ed. M Appel, pp. 437-43. Amsterdam: Elsevier.
- Morner T, Christensson D. 1984. Experimental infection of red foxes (*Vulpes vulpes*) with *Sarcoptes scabiei* var. *vulpes*. *Veterinary Parasitology* 15: 159-64.
- Morner T, Obendorf D, Artois M, Woodford M. 2002. Surveillance and monitoring of wildlife diseases. *Revue Scientifique et Technique (International Office of Epizootics)* 21: 67-76.
- Mukapuli A. 2004. *Cape fur seal pup production and trends in Namibia*. Report. Ministry of Fisheries and Marine Resources, Lüderitz.
- Muller G, Kirk R, Scott D, Miller W, Girffin C. 2001. *Muller & Kirk's Small Animal Dermatology*. Philadelphia: W.B. Saunders. 1528 pp.
- Muller G, Siebert U, Wunschmann A, Artelt A, Baumgartner W. 2000. Immunohistological and serological investigation of morbillivirus infection in harbour porpoises (*Phocoena phocoena*) from the German Baltic and North Sea. *Veterinary Microbiology* 75: 17-25.
- Munson L, Marker L, Dubovi EJ, Spencer J, Evermann JF, O'Brien SJ. 2004. Serosurvey of viral infections in free-ranging Namibian cheetahs (*Acinonyx jubatus*). *Journal of Wildlife Diseases* 40: 23-31.
- Munson L, van Vuuren M, Truyen U. 2000. Genetic characterisation of feline parvovirus sequences from various canrnivores. *Journal of General Virology* 81: 345-50.
- Murray D, Kapke C, Evermann J, Fuller T. 1999. Infectious disease and the conservation of free-ranging large carnivores. *Animal Conservation* 2: 241-54.
- Nams V. 1999. *Locate II*, 1.82. Truro.
- Neal A, White G, Bruce Gill R, Reed D, Olterman J. 1993. Evaluation of mark-resight model assumptions for estimating mountain sheep numbers. *Journal of Wildlife Management* 57: 436-50.
- Nel JAJ, Loutit R, Bothma JD. 1997. Prey use by black-backed jackals along a desert coast. *South African Journal of Wildlife Research* 27: 100-4.
- Nel LH, Thomson GR, Vonteichman BF. 1993. Molecular epidemiology of rabies virus in South-Africa. *Onderstepoort Journal of Veterinary Research* 60: 301-6.
- NPC. 2003. *2001 Population and housing census Republic of Namibia. National report: basic analysis with highlights*. National Planning Commission, Central Bureau of Statistics, Windhoek.
- Odendaal J. 1994. Demographics of companion animals in South Africa. *Journal of the South African Veterinary Association* 65: 67-72.
- Oosthuizen W, Meyer M, David J, Summers N, Kotze P, Swanson S. 1997a. Variation in jackal numbers at the Van Reenen Bay seal colony with comment on likely importance of jackals as predators. *South African Journal of Wildlife Research* 27: 26-9.

- Oosthuizen WH. 1991. General movements of South African (Cape) fur seals *Arctocephalus pusillus pusillus* from analysis of recoveries of tagged animals. *South African Journal of Wildlife Research* 11: 21-9.
- Oosthuizen WH, Bester MN. 1997. Comparison of age determination techniques for known-age Cape fur seals. *South African Journal of Zoology* 32: 106-11.
- Oosthuizen WH, Meyer MA, David JHM, Summers NM, Kotze PGH, Swanson SW. 1997b. Variation in jackal numbers at the Van Reenen Bay seal colony with comment on likely importance of jackals as predators. *South African Journal of Wildlife Research* 27: 26-9.
- Osterhaus A. 1988. Seal Death. *Nature* 334: 301-2.
- Osterhaus A, de Swart R, Vos H, Ross P, Kenter M, Barrett T. 1995a. Morbillivirus infections of aquatic mammals: newly identified members of the genus. *Veterinary Microbiology* 44: 219-27.
- Osterhaus A, Deswart RL, Vos HW, Ross PS, Kenter MJH, Barrett T. 1995b. Morbillivirus infections of aquatic mammals - newly identified members of the genus. *Veterinary Microbiology* 44: 219-27.
- Osterhaus A, Groen J, UytdeHaag F, Visser I, van de Bildt M, Bergman A. 1989. Distemper virus in Baikal seals. *Nature* 338: 209-10.
- Osterhaus A, van de Bildt M, Vedder L, Martina B, Niesters H *et al.*,. 1998. Monk seal mortality: virus or toxin. *Vaccine* 16: 979-81.
- Osterhaus A, Vedder EJ. 1988. Identification of virus causing recent seal deaths. *Nature* 335: 20-.
- Packer C, Altizer S, Appel MJ, Brown E, Martenson J *et al.*,. 1999. Viruses of the Serengeti: patterns of infection and mortality in African lions. *Journal of Animal Ecology* 68: 1161-78.
- Paetkau D, Slade R, Burden M, Estoup A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* 13: 55-65.
- Pallett J. 2000. *An assessment of seals and sealing in Namibia*. Report. Wildlife Society of Namibia, Windhoek.
- Pareto V. 1906. *Manuale d'Economia Politica*. Milan
- Parish C. 1994. The emergence and evolution of canine parvovirus-an example of recent host range mutation. *Seminars in Virology* 5: 121-32.
- Pence D, Lamar A, Pence B, Sprowls R. 1983. The epizootology and pathology of sarcoptic mange in coyotes, *Canis latrans*, from south Texas. *Journal of Parasitology* 69: 1100-15.
- Pence D, Windberg L. 1994. Impact of a sarcoptic mange epizootic on a coyote population. *Journal of Wildlife Management* 58: 624-33.
- Perry B. 1993. Dog ecology in eastern and southern Africa: implications for rabies control. *Onderstepoort Journal of Veterinary Research* 60: 429-36.
- Peterson R, Thomas N, Thurber J, Vucetich J, Waite T. 1998. Population limitation and the wolves of Isle Royale. *Journal of Mammalogy* 79: 828-41.

- Petrie A, Watson P. 1999. *Statistics for veterinary and animal science*. Oxford: Blackwell Science. 243 pp.
- Philippa J, Leighton F, Daoust P, Neilsen O, Pagliarulo M *et al.*,. 2004. Antibodies to selected pathogens in free-ranging terrestrial carnivores and marine mammals in Canada. *Veterinary Record* 155: 135-40.
- Pollock R. 1982. Experimental canine parvovirus infection in dogs. *Cornell Veterinarian* 72: 103-19.
- Poulin R. 1996. Sexual inequalities in helminth infections: a cost of being male? *American Naturalist* 147: 287-95.
- ProMed-mail. 2002. *Rabies, Antelope, Other animals - Namibia*. <<http://www.promedmail.org>>. ProMed-mail. Accessed: 2004.
- Pursell A, Stuart B, Styer E. 1983. Isolation of an adenovirus from black bear cubs. *Journal of Wildlife Diseases* 19: 269-71.
- Quinn P, Carter M. 1994. Enterobacteria. In *Clinical Veterinary Microbiology*, ed. P Quinn, M Carter, pp. 648. London: Wolfe.
- Raether W, Hanel H. 2003. Epidemiology, clinical manifestations and diagnosis of zoonotic cestode infections: an update. *Parasitology Research* 91: 412-38.
- Randall D, Williams S, Kuzmin I, Rupprecht CE, Tallents L *et al.*,. 2004. Rabies outbreak in endangered Ethiopian wolves. *Emerging Infectious Diseases* 10: 2214-7.
- Rautenbach G, Boomker J, Villiers I. 1991. A descriptive study of the canine population in a rural town in southern Africa. *Journal of the South African Veterinary Association* 62: 158-62.
- Refsum T, Heir E, Kapperud G, Vardund T, Holstad G. 2002. Molecular epidemiology of *Salmonella enterica* serovar *typhimurium* isolates determined by pulsed-field gel electrophoresis: Comparison of isolates from avian wildlife, domestic animals, and the environment in Norway. *Applied and Environmental Microbiology* 68: 5600-6.
- Rhodes C, Atkinson R, Anderson R, Macdonald D. 1998. Rabies in Zimbabwe: reservoir dogs and the implications for disease control. *Philosophical Transactions of the Royal Society, London, Series B: Biol Sciences* 353: 999-1010.
- Riley SPD, Foley J, Chomel B. 2004. Exposure to feline and canine pathogens in bobcats and gray foxes in urban and rural zones of a National Park in California. *Journal of Wildlife Diseases* 40: 11-22.
- Riley SPD, Hadidian J, Manski DA. 1998. Population density, survival, and rabies in raccoons in an urban national park. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 76: 1153-64.
- Rima B, Curran M, Kennedy S. 1992. Phocine distemper virus, the agent responsible for the 1988 mass mortality of seals. *The Science of the Total Environment* 115: 45-55.
- Rodriguez B, Arroyo R, Caro L, Orihel TC. 2002. Human dirofilariasis in Costa Rica - A report of three new cases of *Dirofilaria immitis* infection. *Parasite-Journal De La Societe Francaise De Parasitologie* 9: 193-5.
- Roelke-Parker M, Munson L, Packer C, Kock R, Cleaveland S *et al.*,. 1996. A canine distemper virus epidemic in Serengeti lions. *Nature* 379: 441-5.

- Rosatte RC. 2000. Management of raccoons (*Procyon lotor*) in Ontario, Canada: Do human intervention and disease have significant impact on raccoon populations? *Mammalia* 64: 369-90.
- Roscoe DE. 1993. Epizootiology of canine distemper in New Jersey raccoons. *Journal of Wildlife Diseases* 29: 390-5.
- Ross P. 2002. The role of immunotoxic environmental contaminants in facilitating the emergence of infectious diseases in marine mammals. *Human and Ecological Risk Assessment* 8: 277-92.
- Ross P, DeSwart R, Addison R, VanLoveren H, Vos J, Osterhaus A. 1996a. Contaminant-induced immunotoxicity in harbour seals: Wildlife at risk? *Toxicology* 112: 157-69.
- Ross P, Visser I, Broeders H, van de Bildt M, Bowen W, Osterhaus A. 1992. Antibodies to phocine distemper virus in Canadian seals. *Veterinary Record* 130: 514-6.
- Ross PS, Deswart RL, Reijnders PJH, Vanloveren H, Vos JG, Osterhaus A. 1995. Contaminant-related suppression of delayed-type hypersensitivity and antibody-responses in harbor seals fed herring from the Baltic Sea. *Environmental Health Perspectives* 103: 162-7.
- Ross PS, DeSwart RL, Timmerman HH, Reijnders PJH, Vos JG *et al.*,. 1996b. Suppression of natural killer cell activity in harbour seals (*Phoca vitulina*) fed Baltic Sea herring. *Aquatic Toxicology* 34: 71-84.
- Rothenberg RB, McElroy PD, Wilce MA, Muth SQ. 2003. Contact tracing: comparing the approaches for sexually transmitted diseases and tuberculosis. *International Journal of Tuberculosis and Lung Disease* 7: S342-S8.
- Roughton R, Sweeny M. 1982. Refinements in scent-station methodology for assessing trends in carnivore populations. *Journal of Wildlife Management* 46: 217-29.
- Rowe-Rowe D. 1982. Home range and movements of black-backed jackals in an African montane region. *South African Journal of Wildlife Research* 12: 79-84.
- Rypula K, Chmielewski R, Smielewska-Los E, Klimientowski S. 2002. Phylogenetic similarity of the canine parvovirus wild-type isolates on the basis of VP1/VP2 gene fragment sequence analysis. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health* 49: 142-5.
- Saliki JT, Cooper EJ, Gustavson JP. 2002. Emerging morbillivirus infections of marine mammals: development of two diagnostic approaches. *Annals of the New York Academy of Science* 969: 51-9.
- Saliki JT, Lehenbauer TW. 2001. Monoclonal antibody-based competitive enzyme-linked immunosorbent assay for detection of morbillivirus antibody in marine mammal sera. *Journal of Clinical Microbiology* 39: 1877-81.
- Scheepers JL, Venzke KAE. 1995. Attempts to reintroduce African wild dogs *Lycaon pictus* into Etosha National Park, Namibia. *South African Journal of Wildlife Research* 25: 138-40.
- Schneider H. 1994. *Animal health and veterinary medicine in Namibia*. Windhoek, Namibia: AGRIVET. 272 pp.
- Schoeman A. 1996. Conservation in Namibia: laying the foundation. *Namibia Environment* 1: 6-13.
- Scholin CA, Gulland F, Doucette GJ, Benson S, Busman M *et al.*,. 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* 403: 80-4.



- Scott D, Miller H, Griffin C. 2001. Parasitic skin diseases. In *Miller and Kirk's Small Animal Dermatology*. Philadelphia: WB Saunders Company.
- Scott M. 1988. The impact of infection and disease on animal populations: implications for conservation biology. *Conservation Biology* 2: 40-56.
- Sharmir M, Yakobson B, Baneth G, King R, Dar-Verker S *et al.*, 2001. Antibodies to selected canine pathogens and infestation with intestinal helminths in golden Jackals (*Canis aureus*) in Israel. *The Veterinary Journal* 162: 66-72.
- Sillerio-Zubiri C, King A, Macdonald D. 1996. Rabies and mortality in Ethiopian wolves (*Canis simensis*). *Journal of Wildlife Diseases* 32: 80-6.
- Skidmore PJ, Dooley DP, DeWitt C. 2000. Human extrapulmonary dirofilariasis in Texas. *Southern Medical Journal* 93: 1009-10.
- Sloane M, Sunnucks P, Alpers D, Beheregaray L, Taylor A. 2000. Highly reliable genetic identification of individual northern hairy-nosed wombats from single remotely collected hairs: a feasible censusing method. *Molecular Ecology* 9: 1233-40.
- Smith A, Skilling D. 1979. Viruses and virus diseases of marine mammals. *Journal of the American Veterinary Medical Association* 175: 918-20.
- Smith A, Vedros N, Akers T, Gilmartin W. 1978. Hazards of disease transfer from marine mammals to land mammals: review and recent findings. *Journal of the American Veterinary Medical Association* 173: 1131-3.
- Smith B. 1999. Skeleton of the head and dentition. In *Canine anatomy*, pp. 85-6. Philadelphia: Lippincott Williams & Wilkins.
- Smith HT, Engeman RM. 2002. An extraordinary Raccoon, *Procyon lotor*, density at an Urban Park. *Canadian Field-Naturalist* 116: 636-9.
- Smith JS, Yager PA, Orciari LA. 1993. Rabies in wild and domestic carnivores of Africa - epidemiologic and historical associations determined by limited sequence analysis. *Onderstepoort Journal of Veterinary Research* 60: 307-14.
- Soma T, Hara M, Ishii H, Yamamoto S. 2001. Antibody testing against canine coronavirus by immunoperoxidase plaque staining. *Veterinary Research Communications* 25: 327-36.
- Soma T, Ishii H, Hara M, Ohe K, Hagimori I *et al.*, 2003. Detection of canine distemper virus antigen in canine serum and its application to diagnosis. *Veterinary Record* 153: 499-501.
- Sorin C, Mvula J. 2001. *Rabies study: In north central division, Namibia*. Report. Directorate of Veterinary Services, Ondangwa.
- Spencer JA, Bingham J, Heath R, Richards B. 1999. Presence of antibodies to canine distemper virus, canine parvovirus and canine adenovirus type 1 in free-ranging jackals (*Canis adustus* and *Canis mesomelas*) in Zimbabwe. *Onderstepoort Journal of Veterinary Research* 66: 251-3.
- Stanton JB, Brown CC, Poet S, Lipscomb TP, Saliki JT, Frasca S, Jr. 2004. Retrospective differentiation of canine distemper virus and phocine distemper virus in phocids. *Journal of Wildlife Diseases* 40: 53-9.
- Stanton JB, Poet S, Frasca S, Jr., Bienzle D, Brown CC. 2002. Development of a semi-nested reverse transcription polymerase chain reaction assay for the retrospective diagnosis of canine distemper virus infection. *Journal of Veterinary Diagnostic Investigation* 14: 47-52.



- Steinel A, Venter E, Van Vuuren M, Parrish C. 1998. Antigenic and genetic analysis of canine parvoviruses in Southern Africa. *Onderstepoort Journal of Veterinary Research* 65: 239-42.
- Stephenson R, Ritter D, Nielsen C. 1982. Serologic survey for canine distemper and infectious canine hepatitis in wolves in Alaska. *Journal of Wildlife Diseases* 18: 419-24.
- Stuart C. 1976. Diet of the black-backed jackal *Canis mesomelas* in the central Namib Desert, South West Africa. *Zoologica Africana* 11: 193-205.
- Stuart C, Shaughnessy P. 1984. Content of *Hyaena brunnea* and *Canis mesomelas* scats from southern coastal Namibia. *Mammalia* 48: 611-2.
- Sun SJ, Bennett DE, Flood J, Loeffler AM, Kammerer S, Ellis BA. 2002. Identifying the sources of tuberculosis in young children: A multistate investigation. *Emerging Infectious Diseases* 8: 1216-23.
- Sutherland W, ed. 1996. *Ecological census techniques: a handbook*. University of East Anglia: Cambridge University Press. 336 pp.
- Swanepoel R, Barnard BJ, Meredith CD, Bishop GC, Bruckner GK *et al.*,. 1993a. Rabies in southern Africa. *Onderstepoort J Vet Res* 60: 325-46.
- Swanepoel R, Barnard BJH, Meredith CD, Bishop GC, Bruckner GK *et al.*,. 1993b. Rabies in Southern Africa. *Onderstepoort Journal of Veterinary Research* 60: 325-46.
- Swinton J, Harwood J, Grenfel B, Gilligan C. 1997. Persistence thresholds for phocine distemper virus infection in harbour seal *Phoca vitulina* metapopulations. *Journal of Animal Ecology* 66
- Tarello W. 2002. Cutaneous lesions in dogs with *Dirofilaria (Nochtiella) repens* infestation and concurrent tick-borne transmitted diseases. *Veterinary Dermatology* 13: 267-74.
- Taylor A, Alpers D, Sherwin W. 1998. Remote censusing of Northern hairy-nosed wombats *Lasiorninus krefftii* via genetic typing of hairs collected in the field. In *Wombats*, ed. R Wells, P Pridmore, pp. 156-64. Chipping Norton: Surrey Beatty & Sons.
- Taylor L, Latham S, Woolhouse M. 2001. Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society, London, Series B: Biol Sciences* 356: 983-9.
- Thorne E, Williams E. 1988. Disease and endangered species: the black-footed ferret as a recent example. *Conservation Biology* 2: 66-74.
- Thornton SM, Nolan S, Gulland FMD. 1998. Bacterial isolates from California sea lions (*Zalophus californianus*), harbor seals (*Phoca vitulina*), and northern elephant seals (*Mirounga angustirostris*) admitted to a rehabilitation center along the central California coast, 1994-1995. *Journal of Zoo and Wildlife Medicine* 29: 171-6.
- Thrusfield M. 1995a. Predictive value. In *Veterinary Epidemiology*, pp. 277-80. Edinburgh: Blackwell Science.
- Thrusfield M. 1995b. *Veterinary Epidemiology*. Oxford: Blackwell Science
- Tompkins D, Dobson A, Arneberg P, Begon M, Cattadori I *et al.*,. 2002. Parasites and host population dynamics. In *The Ecology of Wildlife Diseases*, ed. P Hudson, A Rizzoli, B Grenfell, H Heesterbeek, A Dobson, pp. 45-62. Oxford: Oxford University Press.

- Torres P, Cuevas C, Tang M, Barra M, Franjola R *et al.*, 2004. Introduced and native fishes as infection foci of *Dipyllobothrium* spp. in humans and dogs from two localities at Lake Panguipulli in Southern Chile. *Comparative Parasitology* 71: 111-7.
- Truyen U. 1996. Evolution of canine parvovirus involved loss and gain of feline host range. *Virology* 215: 186-9.
- Truyen U, Muller T, Heidrich R, Tackmann K, Carmichael LE. 1998. Survey on viral pathogens in wild red foxes (*Vulpes vulpes*) in Germany with emphasis on parvoviruses and analysis of a DNA sequence from a red fox parvovirus. *Epidemiol and Infection* 121: 433-40.
- Truyen U, Parish C. 1995. The evolution and control of parvovirus host ranges. *Virology* 6: 311-7.
- Tsung SH, Liu CC. 2003. Human pulmonary dirofilariasis in Taiwan. *Journal of the Formosan Medical Association* 102: 42-5.
- Valenzuela D, Ceballos G, Gracia A. 2000. Mange epizootic in white-nosed coatis in western Mexico. *Journal of Wildlife Diseases* 36: 56-63.
- Van Bresseem M, Van Waerebeek K, Raga J. 1999. A review of virus infections of cetaceans and the potential impact of morbilliviruses, poxviruses and papillomaviruses on host population dynamics. *Diseases of Aquatic Organisms* 38: 53-65.
- Van Bresseem MF, Van Waerebeek K, Jepson PD, Raga JA, Duignan PJ *et al.*, 2001. An insight into the epidemiology of dolphin morbillivirus worldwide. *Veterinary Microbiology* 81: 287-304.
- van de Bildt M, Martina B, Sidi B, Osterhaus A. 2001. Morbillivirus infection in a bottlenosed dolphin and a Mediterranean monk seal from the Atlantic coast of West Africa. *Veterinary Record* 148: 210-1.
- van de Bildt M, Martina B, Vedder E, Androukaki E, Kotomatas S *et al.*, 2000. Identification of morbilliviruses of probable cetacean origin in carcasses of Mediterranean monk seals (*Monachus monachus*). *Veterinary Record* 146: 691-4.
- van de Bildt M, Vedder E, Martina B, Abou Sidi B, Jiddou A *et al.*, 1999. Morbilliviruses in Mediterranean monk seals. *Veterinary microbiology* 69: 19-21.
- van de Bildt MW, Kuiken T, Visee AM, Lema S, Fitzjohn TR, Osterhaus AD. 2002. Distemper outbreak and its effect on African wild dog conservation. *Emerging Infectious Diseases* 8: 211-3.
- Van der Merwe N. 1953. The Jackal. *Fauna Flora (Pretoria)* 4: 1-83.
- van Dreyer A, Nel J. 1990. Feeding-site selection by black-backed jackals on the Namib Desert coast. *Journal of Arid of Environments* 19: 217-24.
- Van Heerden J. 1980. The transmission of *Babesia canis* to the wild dog *Lycaon pictus* (Temminck) and black-backed jackal *Canis mesomelas* Schreber. *Journal of the South African Veterinary Association* 51: 119-20.
- Van Zyl B. 1999. *Culling of seals in Namibia: a conservation necessity*. <http://www.iwmc.org/sustain/2ndSymposium/issues/issues-14-1.htm>. IWMC World Conservation Trust. Accessed: 2004.
- Verster A, Collins F. 1966. The incidence of hyatidosis in the Republic of South Africa. *Onderstepoort Journal of Veterinary Research* 33: 49-72.

- Visser I. 1993. *Morbillivirus infections in seals, dolphins and porpoises*. Publication thesis. University of Utrecht. 155 pp.
- Visser I, Grachev M, Orvell C, De Vries P, Broeders H *et al.*,. 1990. Comparison of two morbilliviruses isolated from seals during outbreaks of distemper in north west Europe and Siberia. *Archives of Virology* 111: 149-64.
- Visser IKG, Vanbressem MF, Deswart RL, Vandebildt MWG, Vos HW *et al.*,. 1993a. Characterization of morbilliviruses isolated from dolphins and porpoises in Europe. *Journal of General Virology* 74: 631-41.
- Visser IKG, Vedder EJ, Vos HW, Vandebildt MWG, Osterhaus A. 1993b. Continued presence of phocine distemper virus in the Dutch Wadden Sea seal population. *Veterinary Record* 133: 320-2.
- von Messling V, Harder TC, Moennig V, Rautenberg P, Nolte I, Haas L. 1999. Rapid and sensitive detection of immunoglobulin M (IgM) and IgG antibodies against canine distemper virus by a new recombinant nucleocapsid protein-based enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology* 37: 1049-56.
- Vucetich JA, Creel S. 1999. Ecological interactions, social organization, and extinction risk in African wild dogs. *Conservation Biology* 13: 1172-82.
- Wallinga J, Edmunds W, Kretzschmar M. 1999. Perspective: human contact patterns and the spread of airborne infectious diseases. *Trends in Microbiology* 7: 372-7.
- Wandeler A, Matter H, Kappeler A, Budde A. 1993. The ecology of dogs and canine rabies: a selective review. *Revue Scientifique et Technique (International Office of Epizootics)* 12: 51-71.
- Way J, Horton J. 2004. *Coyote kills harp seal*. [http://www.canids.org/canidnews/7/Coyote\\_kills\\_harp\\_seal.pdf](http://www.canids.org/canidnews/7/Coyote_kills_harp_seal.pdf). The IUCN/SSC Canid Specialist Group. Accessed: 2004.
- White G. 1996a. Noremark: population estimation from mark-resighting surveys. *Wildlife Society Bulletin* 24: 50-2.
- White G. 1996b. *Program NOREMARK Software Reference Manual*. Accessed:
- White G, Garrott R. 1990. *Analysis of wildlife radio-tracking data*. London: Academic Press. 383 pp.
- White P, Harris S, Smith G. 1995. Fox contact behaviour and rabies spread: a model for the estimation of contact probabilities between urban foxes at different population densities and its implications for rabies control in Britain. *Journal of Applied Ecology* 32: 693-706.
- WHO, WSPA. 1990. *Guidelines for dog population management*. World Health Organisation (WHO), World Society for the Protection of Animals (WSPA), Geneva.
- Wickens P, David J, Shelton P, Field J. 1991. Trends in harvests and pup numbers of the South African fur seal: implications for management. *South African Journal of Marine Science* 11: 307-26.
- Wickens PA. 1993. Life expectancy of fur seals, with special reference to the South-African (cape) fur seal. *South African Journal of Wildlife Research* 23: 101-6.
- Wickens PA, Japp DW, Shelton PA, Kriel F, Goosen PC *et al.*,. 1992. Seals and fisheries in South-Africa - competition and conflict. *South African Journal of Marine Science* 12: 773-89.

- Wilkinson D, Smith G. 2001. A preliminary survey for changes in urban fox (*Vulpes vulpes*) densities in England and Wales, and implications for rabies control. *Mammal Review* 31: 107-10.
- Williams E. 2001. Canine distemper. In *Infectious Diseases of Wild Mammals*, ed. E Williams, I Barker, pp. 50-8. London: Manson Publishing Ltd.
- Williams E, Thorne E. 1996. Infectious and parasitic diseases of captive carnivores, with special emphasis of the black-footed ferret (*Mustela nigripes*). *Revue Scientifique et Technique (International Office of Epizootics)* 15: 91-114.
- Williams E, Thorne E, Appel M, Belitsky D. 1988. Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. *Journal of Wildlife Diseases* 24: 385-98.
- Woodroffe R. 1999. Managing disease threats to wild animals. *Animal conservation* 2: 185-93.
- Woodroffe R, Cleaveland S, Courtenay O, Laurenson M, Artois M. 2004. Infectious disease in the management and conservation of wild canids. In *Biology and Conservation of Wild Canids*, ed. D Macdonald, C Sillero-Zubiri, pp. 123-42. Oxford: Oxford University Press.
- Woodroffe R, Ginsberg J, Macdonald D, eds. 1997. *The African wild dog-status survey and conservation action plan*. Gland: IUCN. 166 pp.
- Woodroffe R, Ginsberg JR. 1998. Edge effects and the extinction of populations inside protected areas. *Science* 280: 2126-8.
- Woods L. 2001. Adenoviral diseases. In *Infectious Diseases of Wild Mammals*, ed. E Williams, I Barker, pp. 202-12. London: Manson Publishing Ltd.
- Woolhouse M, Dye C, Etard J-F, Smith T, Charlwood J *et al.*,. 1997. Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of the National Academy of Sciences of the United States of America* 94: 338-42.
- Wyatt T. 1980. Morell's seals. *Journal du Conseil International Pour L'exploration de la Mer* 39: 1-6.
- Wyman M. 1967. The jackals of the Serengeti. *Animals* 10: 79-83.
- Xiaoming W, Tedford R, Van Valkenburgh B, Wayne RK. 2004. Phylogeny, classification, and evolutionary ecology of the *Canidae*. In *Canids: Foxes, Wolves, Jackals and Dogs. Status Survey and Conservation Action Plan*, ed. C Sillero-Zubiri, M Hoffmann, D Macdonald, pp. 8-20. Gland: IUCN/SSC Canid Specialist Group.
- Yom-Tov Y. 1994. Cattle predation by the golden jackal *Canis aureus* in the Golan heights, Israel. *Biological Conservation* 73: 19-22.
- Young T. 1994. Natural die-offs of large mammals: implications for conservation. *Conservation Biology* 8: 410-8.
- Zarnke R, Ballard W. 1987. Serological survey for selected microbial pathogens of wolves in Alaska, 1975-1982. *Journal of Wildlife Diseases* 23: 77-85.
- Zarnke R, Evans M. 1989. Serologic survey for infectious canine hepatitis virus in grizzly bears (*Ursus arctos*). *Journal of wildlife Diseases* 25: 568-73.
- Zumpt F, Ledger J. 1973. Present epidemiological problems of sarcoptic mange in wild and domestic animals. *Journal of the Southern African Wildlife Management Association* 3: 119-20.

Zurbriggen A, Vandeveld M. 1994. The pathogenesis of nervous distemper. *Progress in Veterinary Neurology* 5: 109-16.